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Prothonotary Warbler (Protonotaria citrea) Plumage as an Indicator for Infection: the Relationship between Haemosporidia Infection and Breast Feather Reflectance in a Neotropical Migrant Passerine

Robert Fithian
Virginia Commonwealth University

PROTHONOTARY WARBLER (*PROTONOTARIA CITREA*) PLUMAGE AS AN IDICATOR FOR INFECTION: THE RELATIONSHIP BETWEEN HAEMOSPORIDIA INFECTION AND BREAST FEATHER REFLECTANCE IN A NEOTROPICAL MIGRANT PASSERINE

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at Virginia Commonwealth University.

by

ROBERT CHARLES FITHIAN
Bachelor of Science, The College of William and Mary, 2007

Director: Dr. D. C. Ghislaine Mayer
Assistant Professor, Department of Biology

Virginia Commonwealth University
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List of Abbreviations

µL	Microliter
ANCOVA	Analysis of Covariance
AHY	After Hatch Year
ASY	After Second Year
bp	Base pairs
°C	degree Celsius
DB	Deep Bottom State Park
ddNTPs	Dideoxynucleoside triphosphates
dNTPs	Deoxynucleoside triphosphates
DG	Dutch Gap at Henricus Park
DNA	Deoxyribonucleic Acid
ExoSAP	Exonuclease I – Shrimp Alkaline Phosphatase
HY	Hatch Year
IACUC	Institutional Animal Care and Use Committee
mL	Milliliter
mM	Millimole
mm	Millimeter
ng	Nanogram
nm	Nanometer
PC	Principle Component
PCA	Principle Component Analysis
PCR	Polymerase Chain Reaction
PNWR	Presquile National Wildlife Refuge
pmol	Picomole
PW	Prothonotary Warbler
RNA	Ribose Nucleic Acid
RT-PCR	Reverse Transcriptase Polymerase Chain Reaction
SLEV	St. Louis Encephalitis Virus
SY	Second Year
U	Unit
UV	Ultraviolet
VMCA	Virginia Mosquito Control Association
WNV	West Nile Virus

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Abstract

PROTHONOTARY WARBLER (*PROTONOTARIA CITREA*) PLUMAGE AS AN INDICATOR FOR INFECTION: THE RELATIONSHIP BETWEEN HAEMOSPORIDIA INFECTION AND BREAST FEATHER REFLECTANCE IN A NEOTROPICAL MIGRANT PASSERINE

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Major Director: D. C. Ghislaine Mayer
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Yellow avian plumage is a direct result of carotenoid pigments obtained in a bird's diet and may act as an indicator for individual health, parasite resistance, and status. This study describes breast feather reflectance of adult Prothonotary Warblers (*Protonotaria citrea*) (n=169), insectivorous Neotropical migrant passerines, throughout the Ultraviolet (UV) and human visible light spectra and examines the relationship between Haemosporidia (pathogen causing Avian Malaria) infection and feather reflectance (n=41). Reflectance was characterized using a Principle Component Analysis evaluating Intensity, Brightness, Hue, UV Intensity, UV Brightness, and UV Chroma. UV and visible light reflectance was higher in birds sampled

earlier in the field season (early clutch) ($p=0.0017$ and $p=0.0743$ respectively). There was no relationship between infection and either visible light or hue. However, UV reflectance was lower in infected birds ($p=0.0843$). This study suggests that UV reflectance is an important indicator for the infection status of a Neotropical migrant passerine.

Introduction

From the standpoint of disease range and transmission, avian migration is extremely important. Worldwide, bird populations act as primary reservoirs for zoonotic and wildlife disease and migration dramatically increases the disease range (Smith et al. 1996, Rappole et al. 2000). Various avian species have been proposed as the primary agent for the distribution of pathogens such as *Borrelia burgdorferi* (Lyme Disease, Anderson et al. 1986), West Nile Virus (Work et al. 1955), and Haemosporidia parasites (Avian Malaria, Hubalek 2004). Furthermore, pathogens carried by birds can be transmitted to other animal taxa or indirectly transferred to human populations (Tsiodras et al. 2008).

Protozoan parasites of the *Plasmodium* and *Haemoproteus* genera are the causative agents of Avian Malaria, a disease that is currently limited to bird species (Bennett et al. 1980, Garvin et al. 2006, Hellgren et al. 2004). Similar to human malaria, the parasites that lead to avian malaria are primarily transmitted by mosquito vectors to a vertebrate host where they invade erythrocytes. Avian Malaria is not typically fatal (Siikamaki et al. 1997, Hatchwell et al. 2001); however, symptoms such as anemia and hemorrhaging are likely to increase with additional environmental and behavioral stressors. If the infection is severe enough it often can lead to anemia and eventually death (Atkinson et al. 2000). The entire range of its physiological effects is not entirely known.

Plasmodium relictum is the primary causative agent of Avian Malaria. Other Haemosporidia parasites such as *Plasmodium anasum* and *Plasmodium gallinaceum* may also

cause this disease. These parasites have a very complex life cycle that spans the digestive and circulatory systems of multiple hosts (**Figure 1**). For *P. relictum*, the parasites develop within the midgut of their transmission vector, the mosquito. The parasites mature into sporozoites and migrate into the salivary glands. Because only female mosquitoes bite a host, only females can act as competent vectors for the pathogen. When the female bites a bird, the sporozoites are transferred into either the lymphatic or circulatory system of the host via saliva, where they travel to the liver and infect hepatocytes. When the sporozoites mature, the host cell lyses and merozoites are released into the circulatory system where they infect host erythrocytes.

Infected erythrocytes traveling through the blood stream may be targeted and destroyed by the host immune system. However *Plasmodium*-infected cells undergo cytoadherance, a process in which the infected cells develop adhesive properties on the cell membrane. This allows the host cell to adhere to the inside of vessel walls, thus preventing detection (Nagao et al. 2008). In the erythrocyte, merozoites mature to the ring stage, then the trophozoite stage, and finally the schizont stage. This asexual reproduction terminates with the release of thousands of new merozoites from each schizont during cell lysing. The merozoites can then either continue to reproduce asexually, invading other erythrocytes, or they remain in the bloodstream and develop into gametocytes (Valkiūnas 2005).

Research conducted by Carolyn Pelnik in 2007 and Elena Grillo in 2009 provided evidence that these pathogens infected breeding birds along the James River in Virginia. Both *Plasmodium* and *Haemoproteus* DNA was amplified from the blood samples of a Nearctic Neotropical migrant passerine, the Prothonotary Warbler (Grillo 2009).

The Prothonotary Warbler breeds within riparian habitats in the Eastern United States. Individuals of this species migrate from their wintering grounds in the pathogen-rich tropical areas of South and Central America across the Gulf of Mexico and into the eastern and central United States to breed (Dunn et al. 1997, **Figure 2**). These breeding grounds include areas on

the James River in Virginia. The geographic range of this passerine makes it a potentially important reservoir for many pathogens. They are also unique among warblers in regard to roosting behavior. Unlike most warblers who roost independently, they have been found to roost communally in Panama (Warkinton et al. 1995) and this distinctive behavior may lead to increased contact within populations and therefore increased disease transmission between individuals (Komar et al. 1999).

The ideal nesting and foraging habitats for the Prothonotary Warbler is the forest understory in riparian ecosystems. These preferences are constant across both its wintering and breeding grounds. The warm and wet environment of their tropical wintering locations and breeding sites is the ideal breeding habitat for mosquitoes. Mosquitoes are the primary vector for many parasitic and viral pathogens. This includes mosquitoes of the *Anopheles* and *Culex* genera which are the vectors for the primary parasites that cause Avian Malaria, *Plasmodium relictum* and *Haemoproteus* species. The overlap of Prothonotary Warbler and mosquito habitat increases the chance of pathogen transmission (Komar et al. 1999).

Within the wintering habitat of the Prothonotary Warbler, the Southern House Mosquito (*Culex quinquefasciatus*) is a cyclopropagative vector for Avian Malaria (Whiteman et al. 2005). This particular arthropod species is broadly distributed among tropical and sub-tropical regions (Pumidonming et al. 2005). A separate but similar species, *Anopheles quadrimaculatus*, is found across the breeding range in the eastern United States. It has been proven, experimentally, to be a competent vector for *Plasmodium relictum* (Hunninen 1953, Anopheles 2008) and could be a potentially important vector for the transmission of Avian Malaria.

Prothonotary Warblers arrive on their James River breeding grounds in early April each year. Males appear about two weeks prior to females and establish territories throughout the region. Males are highly aggressive between the time of their arrival and the completion of breeding in mid-July (Petit 1999). During this period of high aggression and competition, males

are extremely active and defend their acquired territory at the slightest indication of intruding competitor Prothonotary Warblers. Female birds arrive on the breeding grounds in late April and choose a male with whom to establish a territory. Male birds display to both males and females by raising their bill, displaying their breast feathers (**Figure 3**) and singing a distinctive "Tsweet-Tsweet-Tsweet." Female Prothonotary Warblers lay up to six eggs in a single clutch and produce 1-2 clutches per breeding season (Petit 1999). When the final egg is laid, the female begins to spend the majority of her time incubating the eggs and will occasionally leave the nest box to forage.

The Prothonotary Warbler is one of only two species of cavity-nesting New World Warblers, or wood-warblers, and is the only cavity nesting wood-warbler in the eastern United States. The other is the Lucy's Warbler (*Vermivora luciae*) which is found in the southwestern United States (Bent 1953, Terres 1980). Wildlife management projects have shown that artificial cavities provided by nest boxes offer excellent nesting sites for birds. These artificial cavities have dramatically increased population size and improved the ability to locate and study individual nesting pairs (C. Blem pers. Comm.).

Both the male and female Prothonotary Warblers have a very conspicuous, yellow breast plumage. The scientific name, *Protonotaria citrea,* was derived from the golden robes of the Protonotarii, Roman Catholic Church officials. They also only molt these breast feathers once per year (Chapman 1907). This single, complete molt occurs after the breeding season and prior to fall migration, between July and August. The timing of this molt allows individuals to replace damaged feathers before the migration to their wintering grounds in South and Central America. Maintaining structurally sound feathers is important across all bird species. Feathers are used for many reasons, including flight, insulation (Veghte et al. 1965), and sensation (Prum 2002). Feathers are also used for communication within avian species (Prum 2002). Intraspecific evaluation of feather reflectance may be used to establish hierarchies (Hanssen et al. 2007) or to

display health or parasite resistance to a potential mate (Freeman-Gallant et al. 2001). In avian species, sexual dimorphism often exists as plumage differences between males and females, where competing males have adapted more ostentatious feather colors and patterns to display their parasite resistance to potential female mates (Freeman-Gallant 2001).

Previous research regarding the relationship between pathogen infection and plumage reflectance has produced mixed results. In 2002, Hill *et al* found that House Finch males and females infected with Coccidia were substantially less red than uninfected individuals in a captive environment. This was attributed to an inability to convert carotenoids obtained in the standardized diet to their plumage (Hill et al. 2002). The conclusion drawn fro this study was that birds in poor condition and with greater parasite loads tend to be duller and have lower carotenoid concentration than those in good condition (Hill et al. 2002). Additionally, the yellow plumage in Yellowhammers (*Emberiza citronella*, Sundberg 1995) and Greenfinches (*Carduelis chloris*, Merila et al. 1999) was found to be brighter in males with lesser infections of *hematozoa*. In contrast, there was no relationship between the plumage brightness of the Common Redpoll (*Carduelis flammea*) and haemoparasite infection (Seutin 1994).

Males with experimentally raised testosterone levels were also more likely to have higher levels of Coccidia infection and became infected at faster rates (Sheldon 1996). These results supported the Immunocompetence Handicap Hypothesis, which states that male birds with elevated testosterone levels have decreased immune response. There may be a trade off in the energy investment between incubation, feeding, and immune function (Sheldon et al. 1996). Previous studies have shown that reduced immune function may diminish feeding rates (Hanssen 2006). Research has also shown that increased reproductive effort leads to decreased immune response (Deerenberg et al. 1997, Hanssen et al. 2005).

Much of the previous research regarding avian plumage has focused on males. However, in 2008, Hanssen et al. conducted a study on the white secondary and secondary covert wing bars

of female Common Eiders (*Somateria mollissima*). Females presented with an immune challenge in the form of Diphtheria-tetanus had significantly reduced wing bar whiteness (Hanssen et al. 2008). These results support the novel ideas that either male birds use the female plumage to evaluate quality, or that the plumage may be an important factor in female-female interactions and the formation of social hierarchies. Additionally, this study showed that feeding rates were reduced in infected individuals.

The white wing-bars in the plumage of the female Common Eider are considered costly ornaments. White feathers degrade faster than melanin-rich brown or black feathers, so it is costly to keep white feathers (Hanssen et al. 2008). The yellow plumage of the Prothonotary Warbler on the other and is considered an amplifying ornament. Yellow plumage is a direct result of carotenoid pigments obtained in a bird's diet (Vershinin 2008). The feathers are a means to display the pigment (Hanssen et al. 2008).

Prothonotary Warblers are completely insectivorous and feed on a variety of invertebrate taxa. A typical diet consists of Coleoptera, Lepidoptera, Hymenoptera, Araneae, and a variety of insect larvae (LeFebvre et al. 1992). Some notable carotenoid-rich insect taxa include Coleoptera, such as the Ladybird beetle (Britton et al. 1977), Lepidopterans (Eichenseer et al. 2002), and Hymenoptera (Hill et al. 2006). When digested and processed, carotenoids are the sole contributor to yellow, orange and red feather pigmentation. Carotenoids have also been shown to function as anticarcinogens, reducing cancer rates in both plants and animals (Vershinin 2008). Carotenoids also function as antioxidants, and as a visual pigment in animals (Vershinin 2008).

The exact mechanism underlying the uptake of carotenoid from insects to their placement within feather structures is unknown in birds (Hill et al. 2006). Removal of the ileum in the intestine of chickens has been shown to reduce carotenoid uptake the most. There appears to be lipid-independent, region-specific absorption mechanisms for carotenoid uptake (Hill et al.

2006). Conversely, high-lipid diets have also been shown to promote carotenoid absorption while high-fiber diets disrupt it (Hill et al. 2006). Regardless of the mechanism, carotenoid pigments found within feathers cannot be internally generated by Prothonotary Warblers and can only be obtained through their diet (Hill et al. 2006).

Feather reflectance is produced by both pigment and structural tissues that make up the feather barbs. Structural reflectance arises due to the scattering of light reflected by a matrix of keratin and trapped air within the barbs of feathers (Prum et al. 1998). Bright yellow reflectance is both a result of carotenoid pigments and structural aspects of feathers. Because carotenoids have low reflective properties and high absorptive properties, droplets of the pigment deposited within the feather nanostructure must be enhanced by local structural characteristics to maintain highly reflective properties (Shawkey et al. 2005). Carotenoids are deposited in structural white tissues in feathers and the enhanced yellow reflectance comes from additional absorption from the nearby structural tissues. Multiple types of carotenoids exist and changes in red and yellow coloration, particularly in hue and saturation (i.e. color purity), are proportional to changes in types and concentrations of carotenoids in the feather (Hill 2002, Saks et al. 2003).

Carotenoids are also the primary cause of ultraviolet (UV) reflectance in feathers. The presence of UV plumage reflectance and the ability to see into the UV range has been verified within many avian species (Huth et al. 1972, Wright 1972). UV reflection in feathers is produced either structurally through the spongy medullary keratin of feather barbs by coherent scattering, or from pigments, specifically dietary carotenoids (Prum 2003). Differences in UV reflectance in male and female Yellow-breasted Chats could not be explained by carotenoid pigments alone. Surrounding structural tissues likely play an important role in UV reflectance (Shawkey et al. 2005).

UV reflectance is typically higher in white and yellow feathers and lower in darker brown and black plumage. Birds contain a fourth spectrally-distinct photoreceptor specific for UV

wavelengths at 370 nm (Andersson 1999, Bennett 1997). This additional photoreceptor allows for the perception of UV reflectance. Few studies have successfully and thoroughly described UV light reflectance and the factors that may be of influence within an avian population. However, UV reflectance has been shown to be a predictor of male mate choice by females in both European Starlings (Bennett 1997) and Zebra Finches (Bennett 1996).

The Prothonotary Warbler is an excellent study organism in regards to this research project. The Prothonotary Warbler Nest Box Project has established several viable populations for sampling and as a Neotropical migrant, this Virginia breeding species is exposed to areas of high pathogen transmission in their wintering grounds. Because of the dissimilar stresses on male and female birds, the relationship between Haemosporidia infection and breast feather reflectance may vary between the sexes. Additionally, both the male and female birds have conspicuous yellow plumage, which is experimentally proven to have high ultraviolet reflection. An intersexual comparison of reflectance may present interesting results.

Objectives

The primary objectives of this study are to describe how Prothonotary Warbler breast feather reflectance varies across sex, research site, age, and early and late clutches, as well as to evaluate the relationship between Haemosporidia infection and breast feather reflectance (UV, visible light, and hue) in male and female Prothonotary Warblers.

Study System

Prothonotary Warblers were sampled from three breeding sites along the James River in eastern Virginia, United States: Presquile National Wildlife Refuge (PNWR), Dutch Gap Conservation Area (DG), and Deep Bottom State Park (DB). **Figure 4** shows the layout of these three research sites and the distribution of nest boxes within these sites. These sites are three of the five research areas that comprise the Prothonotary Warbler Nest Box Project which currently involves the monitoring of more than 600 avian nest boxes. The project was initiated and

developed by Dr. Charles Blem and Mrs. Leann Blem in 1987. Over the project's 22 year history, roughly 15,000 birds have been banded.

Field Methods

Male Prothonotary Warblers typically arrive on their final breeding locations in early April. In late March, the three study sites were monitored weekly for activity among males. Once substantial territoriality was documented, sampling began. Sampling of Prothonotary Warblers took place at or around high tide on days with tolerable weather conditions. High winds prevented access to Prothonotary Warbler nest boxes and cold temperatures placed birds under unhealthy stress levels when handled by researchers. In 2008, male and female birds were caught during the breeding season between 13 April and 9 July. Beginning in April, if males continually sang or flew within the territory, a capture attempt was made in that area. Utilizing the aggressive behavior of male birds, six meter mist nets were placed within a male's territory. A decoy Prothonotary Warbler male and playback of a recorded Prothonotary Warbler male song was used to lure the targeted birds into the net. On rare occasions females were also caught in mist nets.

Females appeared on the James River breeding grounds after males. When a full clutch was laid, they were captured with hand-held nets from their respective nest boxes. The nest boxes have dimensions of 28 cm x 9 cm x 6 cm with 3.2 cm diameter hole (Blem and Blem 1991). Nest boxes were purposefully placed over water facing inland upon metal conduits. Situated here, canoes could silently and inconspicuously approach nest boxes. This placement helped increase accessibility to the Prothonotary Warbler nests and reduce predation.

Observational data as well as physical measurements and samples were collected from each bird over two field seasons in the springs of 2008 and 2009. The date and time as well as the site location, nest box number, sex, mass, and band number were recorded. Physical measurements included mass (g), wing cord (mm), tail length (mm), and the number of white tail

spots. Each individual was aged and tagged with a U.S. Fish and Wildlife Service bird band (Banding permit 23486). Male Prothonotary Warblers captured on the PNWR and DB were also banded with distinct combinations of color bands to identify previously captured individuals at a distance.

Age was determined by visually contrasting primary and secondary coverts (Pyle 1997). Birds were assigned the age of ASY (after second year) if primary and secondary coverts showed similar coloration. Birds were assigned the age of SY (second year) if the coverts showed marked differences. In cases of uncertainty, an age of AHY (after hatched year) was assigned. The ages of previously banded birds were verified using yearly banding records. Samples were also identified by the date they were taken. Samples collected prior to June 1 were designated as early clutch and any samples collected on June 1 or later were designated as late clutch.

Prothonotary Warblers molt once a year immediately after the breeding season. Once the new feathers are generated and fully formed, circulation is cut off and the feathers are henceforth dead structures (Gill 2007). In order to best explore the relationship between pathogen infection and the breast plumage present at mate selection, breast feathers must be compared to the infection present at feather development. Therefore, blood samples must be taken one breeding season and feathers must be sampled during the following season. Utilizing this method resulted in sampling Prothonotary Warblers over the course of two consecutive breeding seasons in 2008 and 2009. Feather data was first analyzed in 2008 for preliminary data on how site, sex, clutch, and age might influence feather reflectance. This analysis tested feathers sampled in 2008 against 2008 independent variables. In 2009, feather samples were collected from recaptured birds from the 2008 season with known infection presence. The feathers sampled in 2009 were tested against the 2008 infection in order to determine the relationship between infection and avian plumage.

During the 2008 breeding season, breast feather and blood samples were taken from every individual Prothonotary Warbler. During the 2009 breeding season, breast feathers were sampled only from recaptured birds from 2008. In 2008, male and female birds were caught during the breeding season between 13 April and 9 July. Individual and population Haemosporidia infection rates as well as breast feather characteristics were determined from blood samples (see methods below). In 2009, attempts were made to recapture Prothonotary Warblers with established infection results from 2008 and again breast feathers were sampled. The 2009 sampling period took place between 10 April and 29 June. Breast feathers sampled in 2009 were analyzed using the same methods as in 2008.

Breast feather collection involved removing at least 9 individual feathers from the breast of each Prothonotary Warbler using tweezers. Sampling methods varied across the two breeding seasons. In 2008, the first sampling season, breast feathers were taken in a randomized order from various locations across the bird's breast. In 2009, to standardize sampling, equal numbers of feathers were taken from four discrete spots on the bird's breast (**Figure 12)**. Feathers were measured for six reflectance characteristics: Intensity, Brightness, Hue, UV Intensity, UV Brightness, and UV Chroma.

Blood samples were taken from the brachial vein. To prepare the vein, alcohol swabs exposed and cleaned the apteria before a 27 gauge needle drew blood. 10-40 μL of whole blood was collected in heparinized capillary tubes for molecular analysis. Whole blood was transferred to 0.2 mL PCR tubes and placed on ice during the completion of the field work. Once in the lab, whole blood was centrifuged to separate the sera from the pellet and both were stored at -80.0 degrees Celsius.

Blood was also spotted on individual Whatman® FTA filter cards and stored at room temperature for future DNA amplification. FTA cards lyse red blood cells and stabilize nucleic acids, the building blocks of DNA, by protecting them from nucleases as well as UV and

oxidative damage. Concordantly, the cards denature proteins and inactivate organismal and bacterial growth. This includes ceasing the growth of blood-born pathogens within the blood sample and preserving the magnitude of the Haemosporidia infection (Whatman® Website). Sanitized cotton balls were applied with pressure over the wound until bleeding ceased. These procedures were performed as a sub-permitee under the federal and state licenses of Ms. Cathy Viverette of Virginia Commonwealth University (IACUC Permit AM10230).

Laboratory Methods

In 2008, Breast feather collection involved randomly removing 9 feathers from the breast of each Prothonotary Warbler using tweezers. Breast feather analysis was measured using a PX-2 pulsed xenon lamp photo-spectrometer and software OOIColor/OOIIrad (version 2.05.00) from Ocean Optics Inc. This was done in collaboration with Ms. Joanna Hubbard, Ms. Caitlin Kight, and Ms. Leah Wilson in the lab of Dr. John Swaddle at The College of William and Mary. A typical spectrometer output is shown in **Figure 5**. Feather reflectance for each individual was measured by Intensity (peak reflectance between 400-720 nm), Brightness (total light reflectance between wavelengths 400-720 nm), Hue (wavelength at peak reflectance), UV Intensity (peak reflectance from wavelengths 320-400 nm), UV Chroma (proportion of UV reflectance to total reflectance), and UV Brightness (total reflection between 320-400 nm) (Shawkey 2003, Doucet 2003) Further explanation of these reflectance measurements are shown in **Figures 6-11**.

Maintaining dry conditions, feathers were stored in envelopes at room temperature. Nine feathers from each individual were placed one on top of another in order in random order to mimic the breast plumage of a Prothonotary Warbler. The stacked feathers were mounted on a black cardstock background. Five separate spectrometric measurements were taken for each set of feathers and averaged. The spectrometer was set with an integration period of 471 milliseconds, scanning average of ten, and smoothing (pixels) of seven.

In order to recover DNA from FTA cards, five to seven 1.2 millimeter disks were punched from the source card and following a Whatman® protocol, washed with a simplified elution process. In a PCR tube, the cards were washed with 200 µL of the FTA Purification Reagent and incubated at room temperature for five minutes. This step was repeated two additional times for a total of three washes. These washes removed heme proteins and other PCR inhibitors. The punches were then washed with 200 µL of Tris-EDTA Buffer (10mM Tris-HCl, 0.1 mM EDTA, pH 8.0) and incubated for five minutes at room temperature. This step was repeated one additional time for a total of two washes. After each wash, the effluent was removed using filtered pipette tips and discarded, leaving the punches in the tube. After a total of five washes, the punches were air dried in the same tube for eight hours and stored at 4 ^{o}C.

The final elution process was performed under a subsequent Whatman® protocol and completed the DNA extraction. First, 35 µL of a highly basic solution (0.1N NaOH, 0.3mM EDTA, pH 13) was added to each of the PCR tubes containing the dried disks. This solution incubated for five minutes before adding 65 µL of a neutral solution (0.1M Tris-HCl, pH 7.0), stabilizing the eluted nucleic acids. Each tube was then flash-vortexed five times, incubated for 10 minutes, and then flash-vortexed an additional ten times.

Prior to PCR amplification, a NanoDrop (Thermo Scientific, Inc.) was used to determine the nucleic acid and DNA (ng/µL) concentration of each eluted sample. From the determined DNA concentration values, varying volumes were added to a PCR cocktail in order to standardize the amount of DNA used. A concentration of 100 ng per 25 µL of DNA was used in each PCR amplification.

PCR amplification of wood warbler mitochondrial DNA was performed in order to validate successful isolation of DNA from Prothonotary Warblers. This process amplifies a segment of the *cytochrome b* gene specific for that group of warblers and prevents false negatives during future assays. This amplification used forward primer Lswu18F (5'-TTG CTG

AAA GAA GTA CTA AGA-3') and reverse primer Lswu18R (5'-CTG TTT GCA GGA TAT GTA TAC-3') (Winker et al. 1999). PCR amplification was done in 0.2 mL PCR tubes. 25 µL PCR cocktails were comprised of 20 pmol of each primer, 1.25 mM dNTPs, 50mM KCl Buffer (with 1.5mM $MgCl_2$ and 10mM Tris-HCl pH8.8), 0.5 units Taq Polymerase (Bioline, Inc.), and 100 ng of template DNA. The final volume of 25 µL for each reaction was completed with DNase RNase Free Water.

Using this cocktail, the targeted 240 base pair DNA fragment was amplified using an Eppendorf Thermal Cycler. Cycling conditions were optimized for Prothonotary Warblers at an initial denaturation step 94 ^{o}C for 3 min 45 s, followed by 30 cycles at 95 ^{o}C for 1 min, 54.8 ^{o}C for 30 s, and 72 ^{o}C for 30 s and an extended elongation period at 72 ^{o}C for 5 min. The product was then maintained at 4^{o} C. The amplified DNA fragments were visualized by agarose gel electrophoresis on a 1.2 % agarose gel containing pre-mixed ethidium bromide.

Once the presence of warbler DNA was confirmed, samples were assayed for the presence of infection by Haemosporidia. Primers used for the PCR amplification were specified for the cytochrome *b* gene present in the mitochondrial DNA of the Haemosporidia parasites. The cytochrome b gene is highly conserved among apicomplexans. This amplification used forward primer Haem F (5'-ATG GTG CTT TCG ATA TAT GCA TG-3') and reverse primer Haem R2 (5'-GCA TTA TCT GGA TGT GAT AAT GGT-3') to target a gene sequence 520 base pairs long (Waldenström et al. 2004). Ready-to-Go PCR beaded tubes (GE Healthcare, Inc.) were used to complete the PCR reaction. The beaded tubes contain 2.5 units PureTaq DNA Polymerase, 10 mM Tris HCl pH 9, 50 mM KCl, 1.5 mM $MgCl_2$, and 200 µM of dATP, dCTP, dGTP, and dTTPs. The total volume of the reaction was 25 µL. The final components of the reaction were added as follows: DNase RNase Free water, 20 pmol of each primer, and100 ng of template DNA. The use of these beads in this reaction minimized risk of reagent contamination and allowed for more efficient sample processing.

Cycling conditions were optimized for the Haemosporidia DNA with an initial denaturation step of 94 °C for 3 min, followed by 35 cycles of 94 °C for 30 s, 50 °C for 30 s, and 72 °C for 45 s, and an extended elongation period at 72 °C for 10 minutes. The amplified PCR product was maintained at 4 °C. The amplified Haemosporidia PCR product was analyzed on a 2.0% agarose gel containing ethidium bromide. A band was present at approximately 520 base pairs (Waldenström et al. 2004).

The first lane of each observed gel contained a positive control. The positive control was from a bird sampled during the summer of 2007 that was known to be infected with both *Haemoproteus* and *Plasmodium*, two genera of Haemosporidia (D.C. Ghislaine Mayer et al. unpubl. data). The positive control was obtained from graduate student Sarah Knowles and her advisor, Dr. Benjamin Sheldon at Boston College (B. Sheldon pers. comm.).

All lab work was conducted in the Microbial Biology Lab of Dr. D.C. Ghislaine Mayer's at Virginia Commonwealth University.

Statistical Analysis

Statistical analyses were performed using JMP 8.0.1 software. A principle component analysis (PCA) evaluated the six feather reflectance measurements (Intensity, Brightness, Hue, UV Intensity, UV Brightness, and UV Chroma) and condensed the variability in breast feather reflectance to principle components. An analysis of covariance (ANCOVA) then assessed the relationship between a single principle component describing feather reflectance for feathers sampled in 2008 and independent variables such as age (ASY or SY), sex, clutch (early or late), and research site. The ANCOVA accounts for any confounding effects between the independent variables. Once relationships were established between each principle component and the independent variables, a second ANCOVA was performed. This test evaluated the relationship between principle components from feathers sampled in 2009 and infection and any significant relationship revealed in 2008 data.

Results

During the 2008 breeding season, a total of 169 birds were captured and sampled. A complete list of sample data is provided in **Appendix I**. In 2009, 41 birds from the 2008 sampling were recaptured (**Appendix II**). Sample sizes are listed in **Table 1**.

In order to understand the relationship between each of the six feather reflectance characteristics, correlation coefficients were generated for both years (**Table 2**). Due to the high degree of correlation among feather reflectance variables, we conducted a principle component analysis (PCA) to simplify the data. A PCA run on the six reflectance measurements for feathers sampled in 2008 condensed the data into three main principle components (PC) that explain 99.2% of the variability in the data (**Table 3**). PC1 explained 66.5% of the variability in the data and seems to be a good measure of UV reflectance (**Table 4**). PC2 explained 18.1% and seems to be a good measure of Intensity and Brightness (**Table 4**). Intensity and Brightness are quantitative characteristics for the human visible spectrum (visible light characteristics). PC3 explained 14.6% and seems to be a good measure of Hue (**Table 4**).

These three principle components were each independently tested against research site, clutch, age, and sex in order to determine if these variables describe any variation in feather reflectance. An ANCOVA was used to analyze the relationships. Feathers collected from birds in early clutch samples had significantly more UV reflectance (PC1) than late clutch samples ($p=0.0017$) and there was a moderately significant difference between feathers collected from older (ASY) birds compared to younger (SY) bird samples ($p=0.0537$) (**Table 5** and **Figure 13**). There was a moderately significant difference in visible reflectance (PC2) between early clutch samples and late clutch samples ($p=0.0743$) (**Table 6** and **Figure 14**). Females had significantly higher Hue values (PC3) than males ($p=0.0055$), and there were significant differences in hue between research sites ($p=<0.0001$). Additionally, there were moderately significant differences in Hue for age and clutch (**Table 7** and **Figure 15**).

In 2008, blood samples were taken and a PCR tested for Warbler DNA and Haemosporidia infection. The product of the amplified Wood Warbler DNA found in Prothonotary Warbler samples is shown in **Figure 16**. The PCR product of the amplified Haemosporidia DNA is shown in **Figure 17**.

In 2009, 41 Prothonotary Warblers were recaptured for which we had previous data on their infection status from 2008. Feathers were collected. Although collected in the 2009 breeding season, these samples were grown in 2008 either in the presence of the Haemosporidia infection or not. A PCA on these data showed similar results as the larger sampled of feathers sampled in 2008. The first three factors explained 99.7% of the variability in the data (**Table 8**). PC1 explained 67.9% of the variability in the data and seems to be a good measure of UV reflectance (**Table 9**). PC2 explained 17.2% and seems to be a good measure of visible light reflectance (**Table 9**). PC3 explained 14.5% and seems to be a good measure of Hue (**Table 9**).

These three principle components based on feathers sampled in 2009 were individually tested against 2008 infection and any other significant variables from initial ANCOVA testing using feathers sampled in 2008. Any previous significance differences in age were not accounted for in this section because all 41 samples were from ASY birds. The feathers sampled from infected birds had lower UV reflectance (PC1) than those sampled from non-infected birds ($p=0.0843$). Early clutch samples had significantly more UV reflectance (PC1) than late clutch samples ($p=0.0260$) (**Table 10** and **Figure 18**). There were no significant differences in visible light reflectance (PC2) and infection or clutch (**Table 11** and **Figure 19**). Likewise, there were no significant differences in Hue based on infection, clutch, research site, or sex (**Table 12** and **Figure 20**).

Discussion

This study produced several newly described and important findings. Preliminary results regarding the relationships between reflectance measurements showed there is a very strong,

positive correlative relationship between Intensity and Brightness as well as between all of the UV measurements (UV Intensity, UV Brightness, and UV Chroma). These relationships indicate that each of the three UV measurements can be independently used to describe UV feather reflectance. Interestingly, Hue had weak correlative relationships with all other measurements. These results, along with the PCA, revealed that there are three distinctly important components of breast feather reflectance in Prothonotary Warblers: UV reflectance, visible light reflectance, and hue.

Clutch seemed to have similar effects on both UV reflectance and visible light reflectance. Samples taken earlier in the breeding season had significantly higher UV and visible light reflectance. Therefore, clutch was taken into account when analyzing the relationship between infection and UV/visible light reflectance. These results may point to a decrease in plumage reflection throughout the breeding season. Further research should account for temporal variation in sampling when evaluating feathers.

There was also a relationship between age and both UV reflectance and Hue. Samples from older (ASY) birds had higher UV reflectance and Hue values than younger (SY) birds. There was no relationship between age and visible light reflectance. All recaptured birds in 2009 were in their third year, so age was not accounted for when testing the relationship between infection and feather reflectance.

There was a strong relationship between research site and Hue. These findings could be very informative. Because each of the reflectance measurements followed to the same pattern regarding the results (PNWR>DG>DB), breast feather reflectance may be an indicator for habitat quality. The yellow plumage of Prothonotary Warblers is a direct result of carotenoid pigments obtained from their completely insectivorous diet. Therefore, Prothonotary Warbler plumage may indicate insect population levels which are often related to environmental quality.

Female Prothonotary Warblers had significantly higher values for Hue than male birds. An equally interesting result was that there was no relationship between sex and either UV or visible light reflectance. This study focused entirely on breast plumage of Prothonotary Warblers. Observationally, it appears that males raise their bills and expose their breast when displaying and singing to females. However, it may also be informative to sample feathers on or near the head of each individual because the bright yellow plumage extends from the breast up through the neck to the head of male birds. Male and female birds did not differ significantly in regards to any reflectance measurement except for hue. This was an intriguing finding, and supports the Prothonotary Warbler species as a sexually monomorphic species. However, different results may be obtained if head feather reflectance was studied. Female birds appear to have a greener head plumage than males and results may be altered.

Hue appeared to show the most response to 2008 variables. There was at least a moderately significant relationship between Hue and all independent variables: sex, site, clutch, and age.

There were no significant relationships between Haemosporidia infection and either visible light reflectance or Hue. However, UV reflectance was significantly lower for infected Prothonotary Warblers. UV reflectance may be an important indicator for the condition of an individual and could be used to evaluate the health of an entire population. Because UV reflectance is partially a result of carotenoid pigments obtained solely from the diet, it can also be used as an environmental indicator in regards to insect populations.

This is further evidence that birds may use plumage reflectance as an honest indicator for their parasite loads. In the case of the Prothonotary Warbler, their plumage color and reflectance in both the visible and UV spectra are reflective of their diet. The more carotenoids a bird ingests by way of insects, the more reflective they are likely to be in both the visible and UV spectra. Individuals may assess the health of others by observing the breast feather reflectance

and potentially gaining knowledge of their foraging capabilities. Because yellow plumage reflectance is a result of the quantity of carotenoid-rich insects ingested by the Prothonotary Warbler, breast feather reflectance may also indicate the quality of the environment.

Of the 41 recaptured Prothonotary Warblers in 2009, 32 were females and 9 were males. Historical theories believe that females choose males based on reflectance. However, because the majority of the recaptured samples were from female birds, these results may point to the presence of mate selection by males as well. Male reproductive success is closely tied to the foraging capabilities of his mate, as she provides much of the food to his offspring. It would be extremely beneficial to choose a healthy, capable, female mate. These results may also point to female-female assessment and interactions and a possible hierarchy between individuals.

Environmental stressors such as breeding, molting, migration, and predation may all play a role in affecting breast feather reflectance. Additionally, female Prothonotary Warblers may need to preserve more energy throughout the breeding season than males. The female's investment of about five eggs per clutch is tremendous when judged against the investment of sperm by a male. Female Prothonotary Warblers also typically make more frequent feeding trips to offspring than do males (Blem and Blem, 2006). Because of the discrepancy in offspring investment, female feather reflectance may be more responsive to pathogen infection. Recent studies have found a possible non-migratory population of Prothonotary Warblers in Louisiana. As a population circumventing migration, infection prevalence and breast feather reflectance results could potentially be very interesting.

Table 1: Sample sizes for each of the independent variables used in the analysis from the 2008 and 2009 field seasons.

Independent Variable	**2008 Sample Sizes (Total n=169)**		
Clutch	Early Clutch: n=90	Late Clutch: n=79	
Site	PNWR: n=64	DG: n=56	DB: n=49
Sex	Male: n=72	Female: n=97	
Age	ASY: n=123	SY: n=46	
	2009 Sample Sizes (Total n=41)		
Clutch	Early Clutch: n=30	Late Clutch: n=11	
Site	PNWR: n=13	DG: n=8	DB: n=20
Sex	Male: n=9	Female: n=32	
Infection	Infected: n=29	Not Infected: n=12	

Table 2: Correlation Coefficients for the six feather characteristics of PW feathers. The bottom left shows feathers sampled in 2008. The top right shows the feathers sampled in 2009. High correlation coefficients exist between Intensity and Brightness as well as each pair-wise UV comparison.

	Intensity	Brightness	Hue	UV Intensity	UV Brightness	UV Chroma
Intensity	-	0.9929	0.0425	0.6328	0.6086	0.4966
Brightness	0.9712	-	0.0237	0.7011	0.6777	0.5738
Hue	0.2236	0.2863	-	0.0907	0.1267	0.1109
UV Intensity	0.5298	0.6668	0.2938	-	0.9920	0.9741
UV Brightness	0.5040	0.6473	0.2591	0.9579	-	0.9818
UV Chroma	0.3584	0.5136	0.3136	0.9622	0.9880	-

Table 3: Principle components established from the six feather characteristics of PW feathers sampled in 2008. PC1 accounts for 66.5% of the variability in the data. The first three principle components account for 99.2% of the total variability in the data.

Principle Component	Eigenvalue	Percent	Cumulative Percent
1	3.991	66.52	66.53
2	1.082	18.03	84.58
3	0.876	14.61	99.18
4	0.026	0.448	99.62
5	0.013	0.228	99.85
6	0.008	0.148	100.0

Table 4: Eigenvectors for a Varimax rotated factor pattern for the first three principle components for feathers sampled in 2008 (n=169). PC1 represents overall ultraviolet reflectance (UV Intensity, UV Brightness, and UV Chroma). PC2 represents overall visible light reflectance (Intensity and Brightness). PC3 represents Hue.

	PC1	PC2	PC3
Intensity	0.204	0.973	0.080
Brightness	0.369	0.917	0.126
Hue	0.149	0.117	0.982
UV Chroma	0.968	0.149	0.155
UV Intensity	0.929	0.340	0.117
UV Brightness	0.938	0.317	0.082

Table 5: Mean values and ANCOVA results for 2008 variables tested against PC1 (UV reflectance) for feathers sampled in 2008.

Variable	Mean			ANCOVA Results
Whole Model				F=3.915; DF=5; p=0.0022
Clutch	Clutch 1: x= 0.243	Clutch 2: x= -0.277		F=10.182; DF=1; p=0.0017
Site	PNWR: x= 0.103	DG: x= -0.010	DB: x= -0.122	F=0.978; DF=2; p=0.3784
Sex	Male: x= -0.059	Female: x= 0.044		F=1.794; DF=1; p=0.1823
Age	ASY: x= 0.118	SY: x= -0.316		F=3.777; DF=1; p=0.0537

Table 6: Mean values and ANCOVA results for 2008 variables tested against PC2 (visible light reflectance) for feathers sampled in 2008.

Variable	Mean			ANCOVA Results
Whole Model				F=1.613; DF=5; p=0.1595
Clutch	Clutch 1: x= -0.120	Clutch 2: x= 0.137		F=3.226; DF=1; p=0.0743
Site	PNWR: x= 0.177	DG: x= -0.066	DB: x= -0.156	F=1.555; DF=2; p=0.2143
Sex	Male: x= 0.103	Female: x= -0.077		F=1.638; DF=1; p=0.2024
Age	ASY: x= -0.024	SY: x= 0.063		F=0.030; DF=1; p=0.8626

Table 7: Mean values and ANCOVA results for 2008 variables tested against PC3 (Hue) for feathers sampled in 2008.

Variable	Mean			ANCOVA Results
Whole Model				F=7.112; DF=5; p=<0.0001
Clutch	Clutch 1: x= -0.078	Clutch 2: x= 0.089		F=1.341; DF=1; p=0.0985
Site	PNWR: x= 0.368	DG: x= -0.023	DB: x= -0.454	F=12.932; DF=2; p=<0.0001
Sex	Male: x= -0.203	Female: x= 0.151		F=7.912; DF=1; p=0.0055
Age	ASY: x= 0.067	SY: x= -0.179		F=3.502; DF=1; p=0.0631

Table 8: Principle components established from the six feather characteristics of PW feathers sampled in 2009. PC1 accounts for 67.9% of the variability in the data. The first three principle components account for 99.7% of the total variability in the data.

Principle Component	Eigenvalue	Percent	Cumulative Percent
1	4.076	67.93	67.93
2	1.035	17.24	85.17
3	0.869	14.48	99.65
4	0.012	0.203	99.85
5	0.006	0.105	99.96
6	0.002	0.041	100.0

Table 9: Eigenvectors for a Varimax rotated factor pattern for the first three principle components for feathers sampled in 2009. PC1 represents overall ultraviolet reflectance (UV Intensity, UV Brightness, and UV Chroma). PC2 represents overall visible light reflectance (Intensity and Brightness). PC3 represents Hue.

	PC1	PC2	PC3
Intensity	0.292	0.956	0.019
Brightness	0.380	0.924	-0.005
Hue	0.061	0.006	0.998
UV Intensity	0.920	0.381	0.032
UV Brightness	0.931	0.351	0.068
UV Chroma	0.970	0.222	0.050

Table 10: Mean values and ANCOVA results for PC1 (UV reflectance) for feathers sampled in 2009 tested against clutch and 2008 Haemosporidia infection.

Variable	Mean		ANCOVA Results
Whole Model			F=3.901; DF=2; p=0.0288
Clutch	Clutch 1: x= 0.191	Clutch 2: x= -0.520	F=5.365; DF=1; p=0.0260
Infection	Infected: x= -0.147	Not Infected: x= 0.354	F=3.143; DF=1; p=0.0843

Table 11: Mean values and ANCOVA results for PC2 (visible light reflectance) for 2008 feathers tested against clutch and 2008 Haemosporidia infection.

Variable	Mean		ANCOVA Results
Whole Model			F=0.006; DF=2; p=0.9942
Clutch	Clutch 1: x= -0.008	Clutch 2: x= 0.021	F=0.005; DF=1; p=0.9433
Infection	Infected: x= -0.008	Not Infected: x= 0.020	F=0.005; DF=1; p=0.9419

Table 12: Mean values and ANCOVA results for PC3 (Hue) for 2008 feathers tested against 2008 Haemosporidia infection and additional 2009 independent variables found significant from the 2008 analysis.

Variable	Mean			ANCOVA Results
Whole Model				F=0.467; DF=5; p=0.7982
Clutch	Clutch 1: x= 0.026	Clutch 2: x= -0.072		F=0.210; DF=1; p=0.6500
Site	PNWR: x= -0.042	DG: x= -0.186	DB: x= 0.101	F=0.157; DF=2; p=0.8551
Sex	Male: x= -0.321	Female: x= 0.090		F=0.988; DF=1; p=0.3271
Infection	Infected: x= -0.084	Not Infected: x= 0.203		F=0.886; DF=1; p=0.3529

Figure 1: The generalized life cycle of Plasmodium relictum from a mosquito vector to an avian host (Valkiūnas 2005).

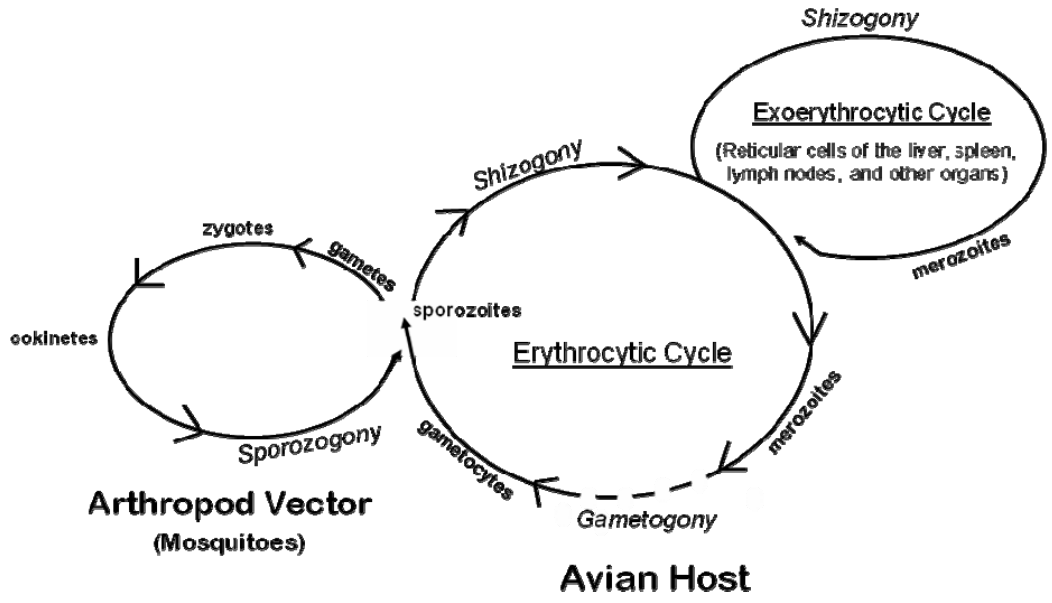


Figure 2: Map of the geographic range of the Prothonotary Warbler. Prothonotary Warblers (adapted from the Cornell Lab of Ornithology) winter in Central and South America and migrate north to the Eastern United States to breed.

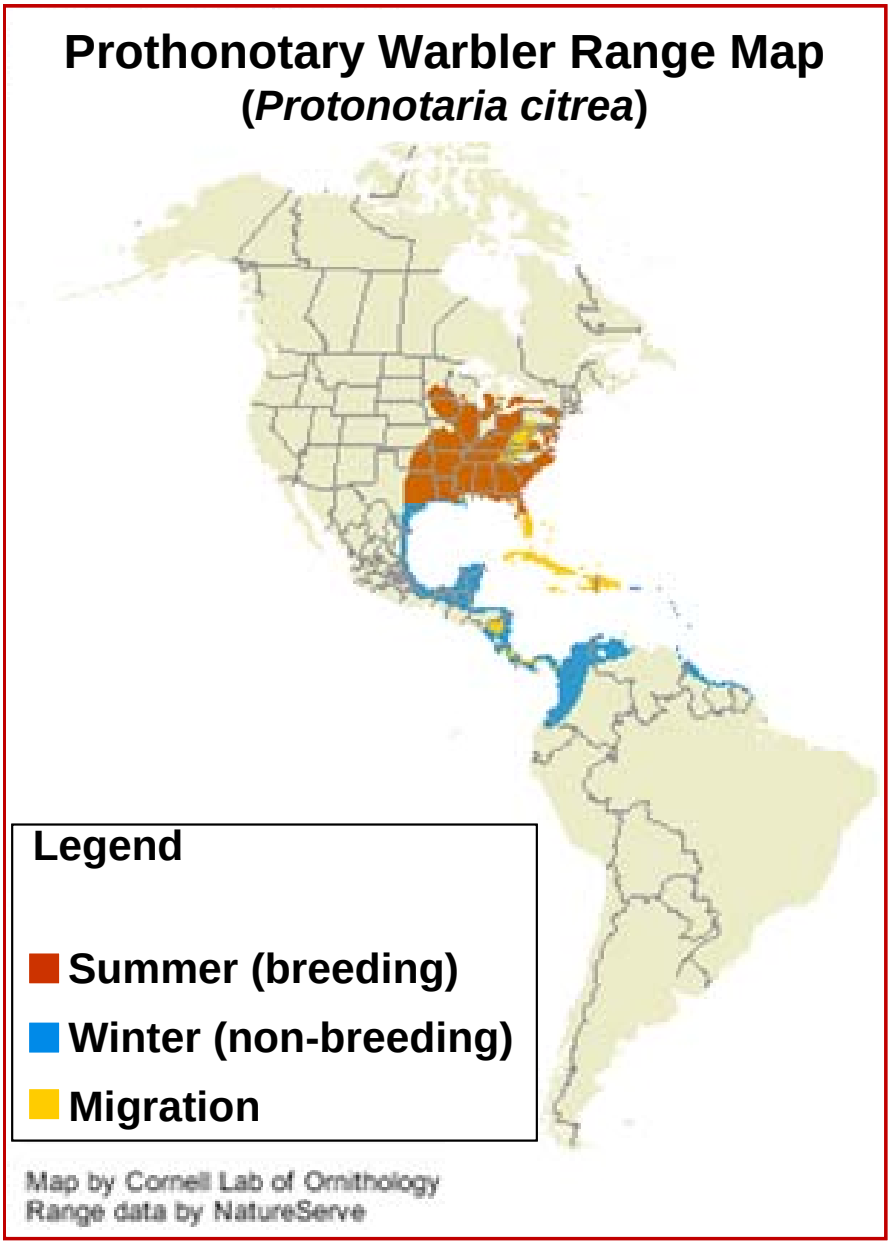


Figure 3: A banded male Prothonotary Warbler (photo taken by Robert Fithian). In a typical display, he raises his bill, displays his breast feathers, and sings. Visual display and song may be used to attract female mates or to establish territories within the breeding site.

Figure 4: A map showing the location and nest box distribution of the three research sites used in this study. All sites were located along the James River just east of Richmond, VA. Colored circles represent nest box placement within the sites.

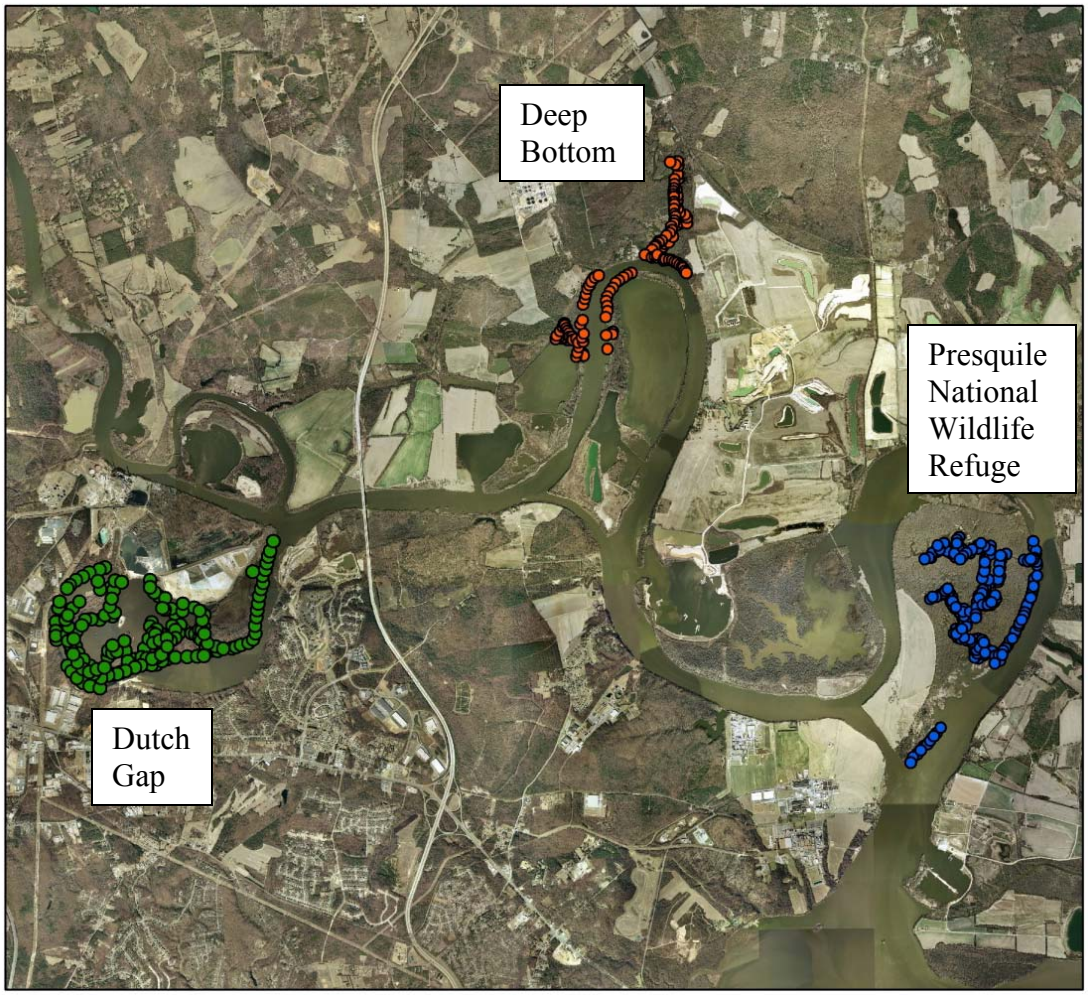


Figure 5: An example of a typical spectrometer output. This graph shows a bimodal reflectance distribution as was found throughout the feather samples.

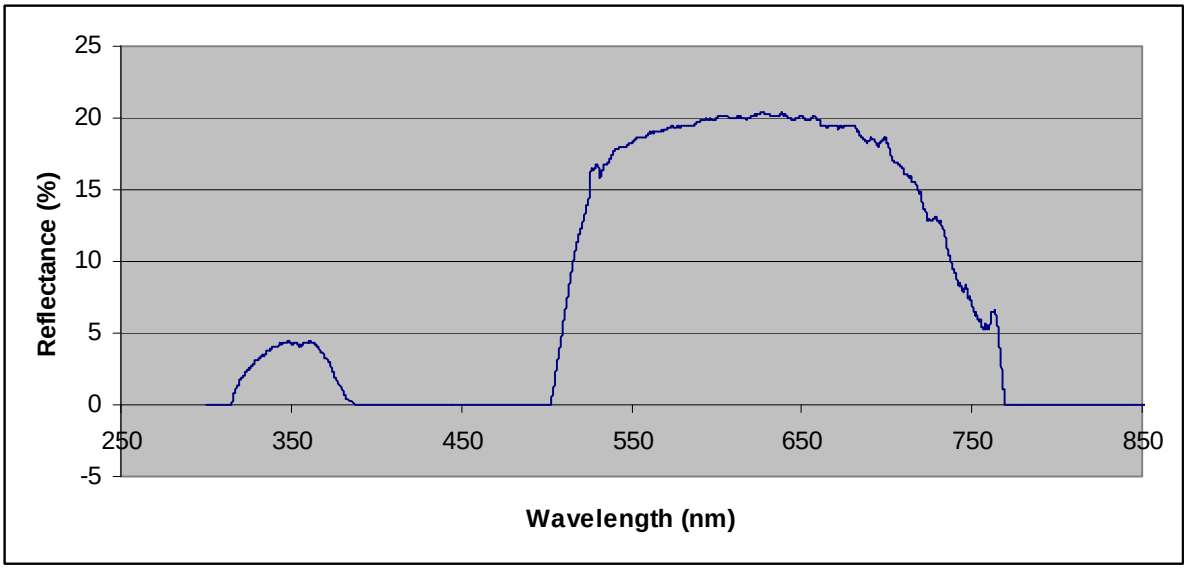


Figure 6: Intensity (peak reflectance between 400-720 nm).

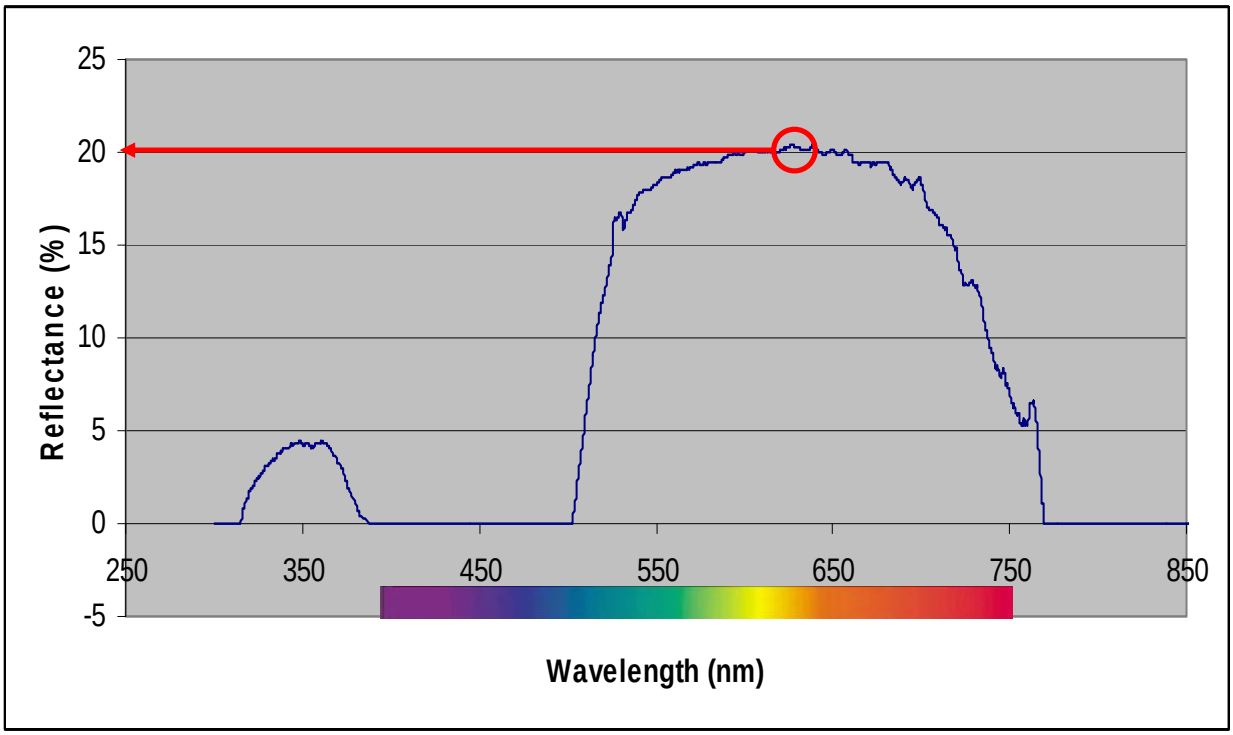


Figure 7: Brightness (total light reflectance between wavelengths 400-720 nm).

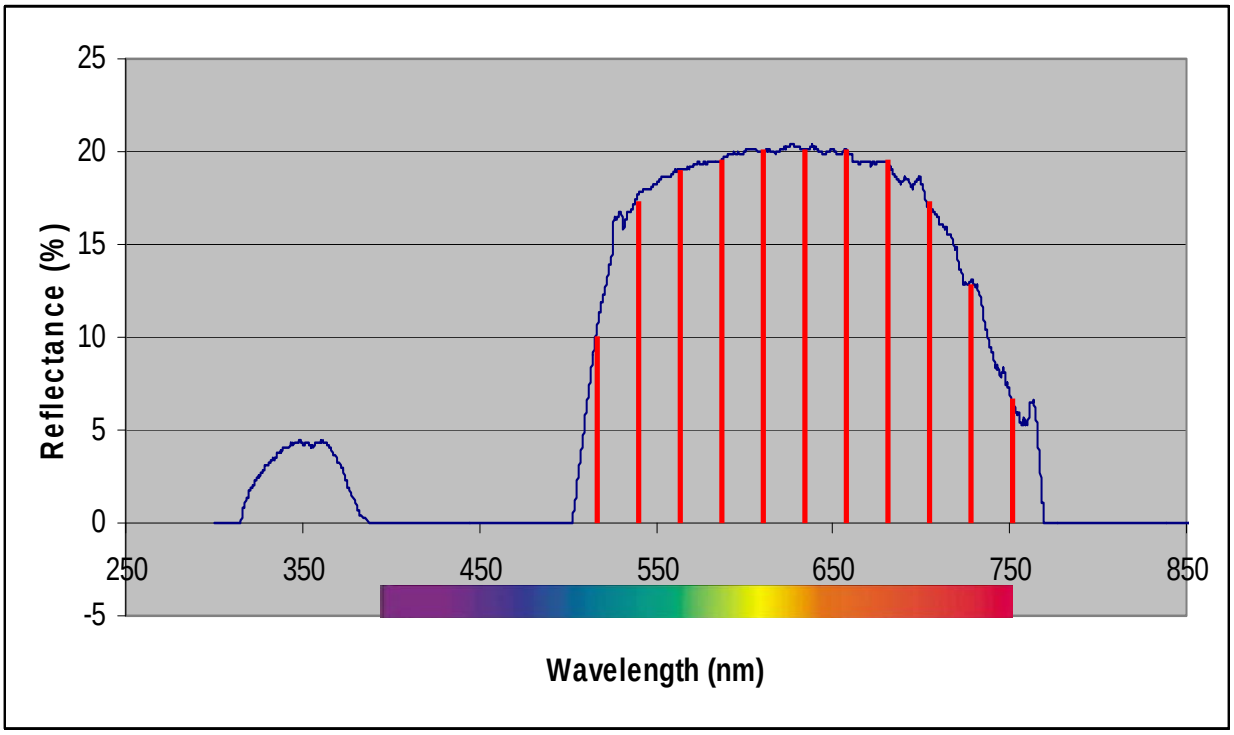


Figure 8: Hue (wavelength at peak reflectance).

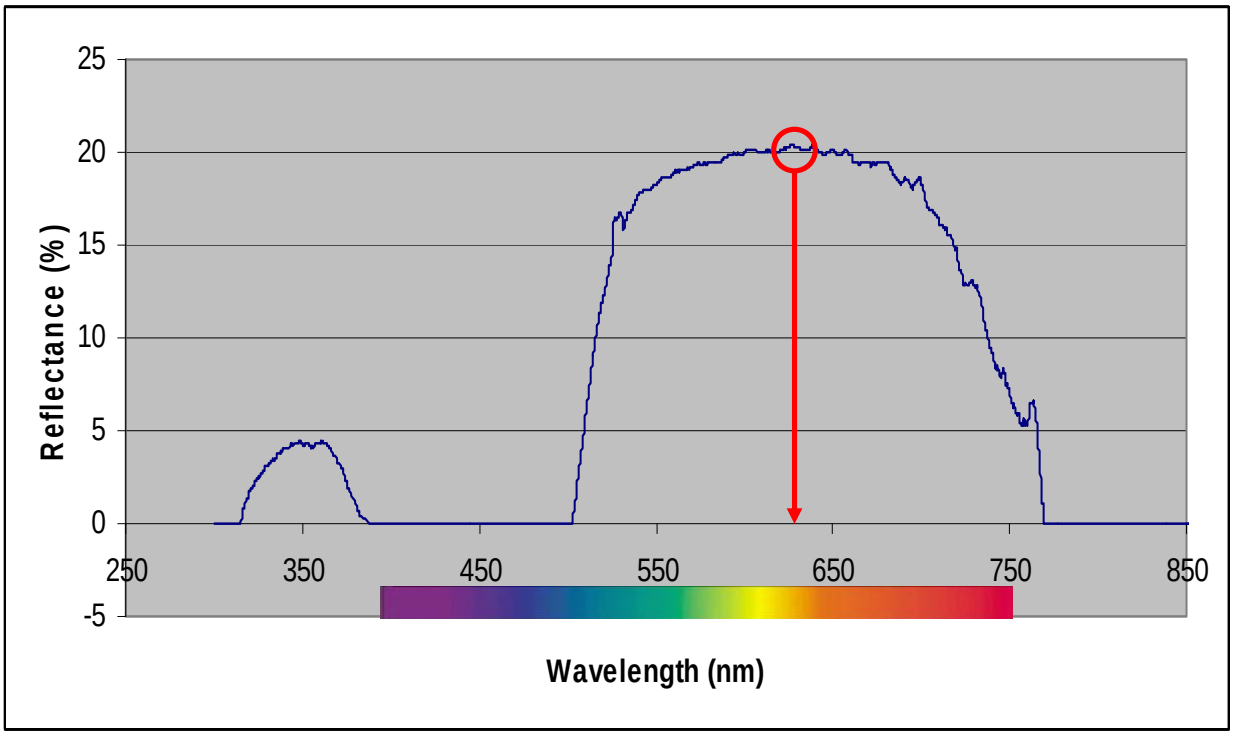


Figure 9: UV Intensity (peak reflectance from wavelengths 320-400 nm).

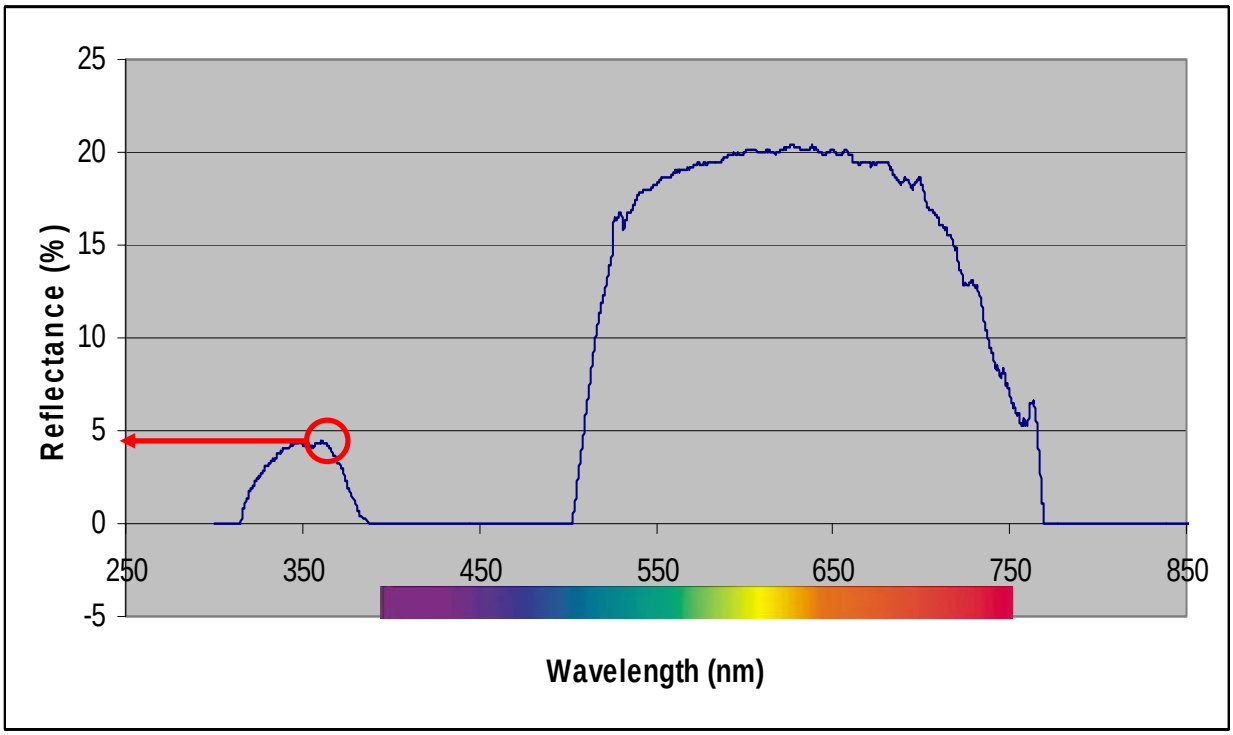


Figure 10: UV Brightness (total reflection between 320-400 nm).

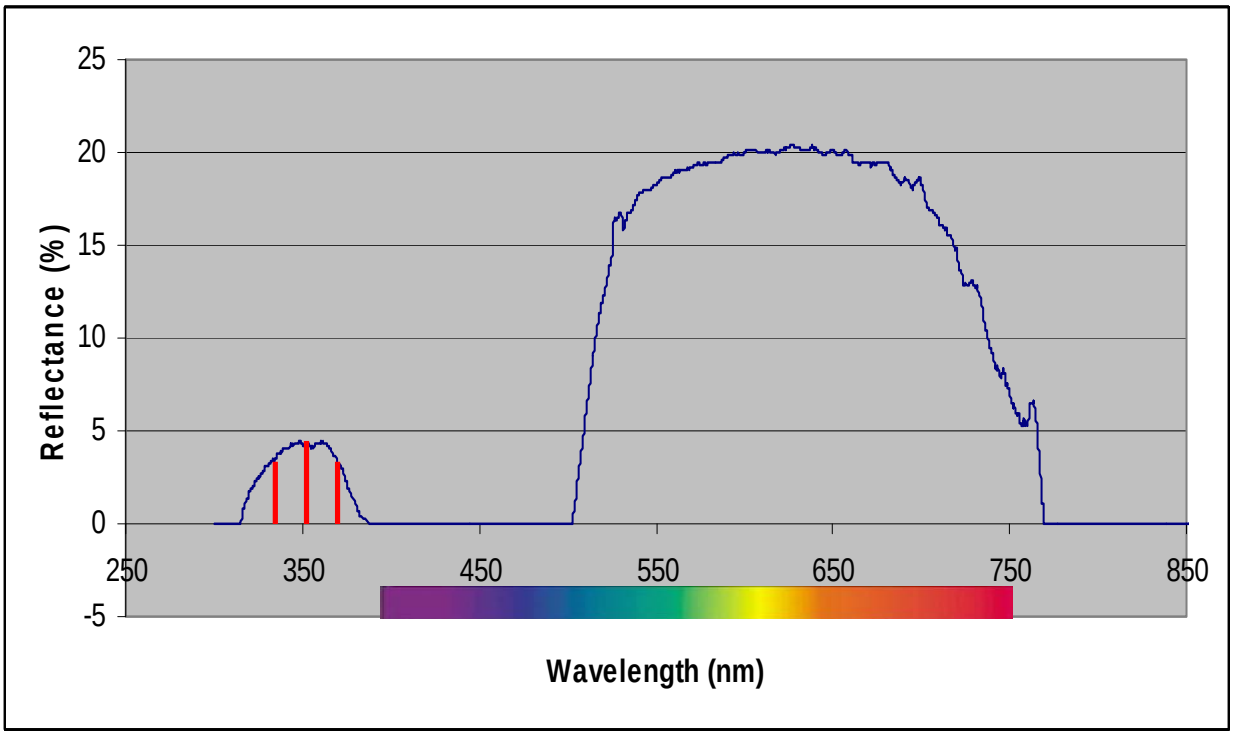
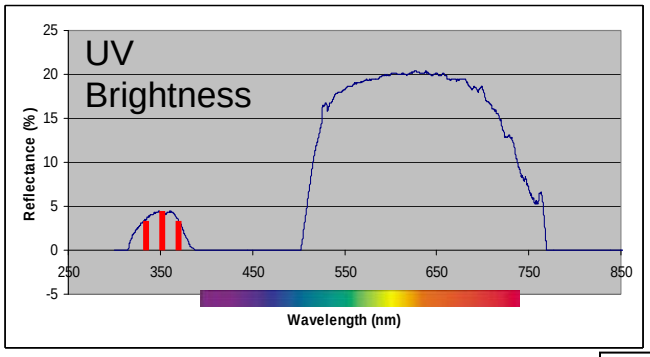


Figure 11: UV Chroma (proportion of UV reflectance to total reflectance).

Proportion of



To

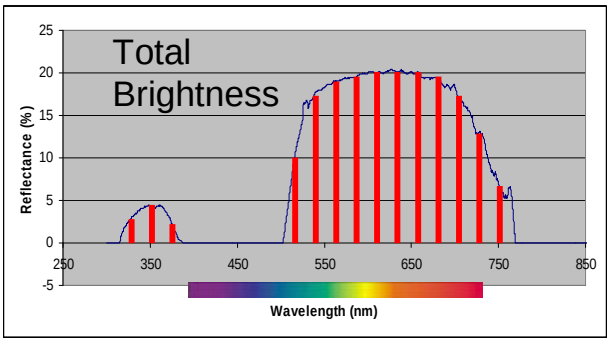


Figure 12: The four sampling areas from which feathers were taken from the Prothonotary Warbler breast. This method was performed during the 2009 breeding season in order to standardize the feather sampling.

Figure 13: Mean PC1 values (representing UV reflectance) for 2008 independent variables: age, clutch, site, and sex.

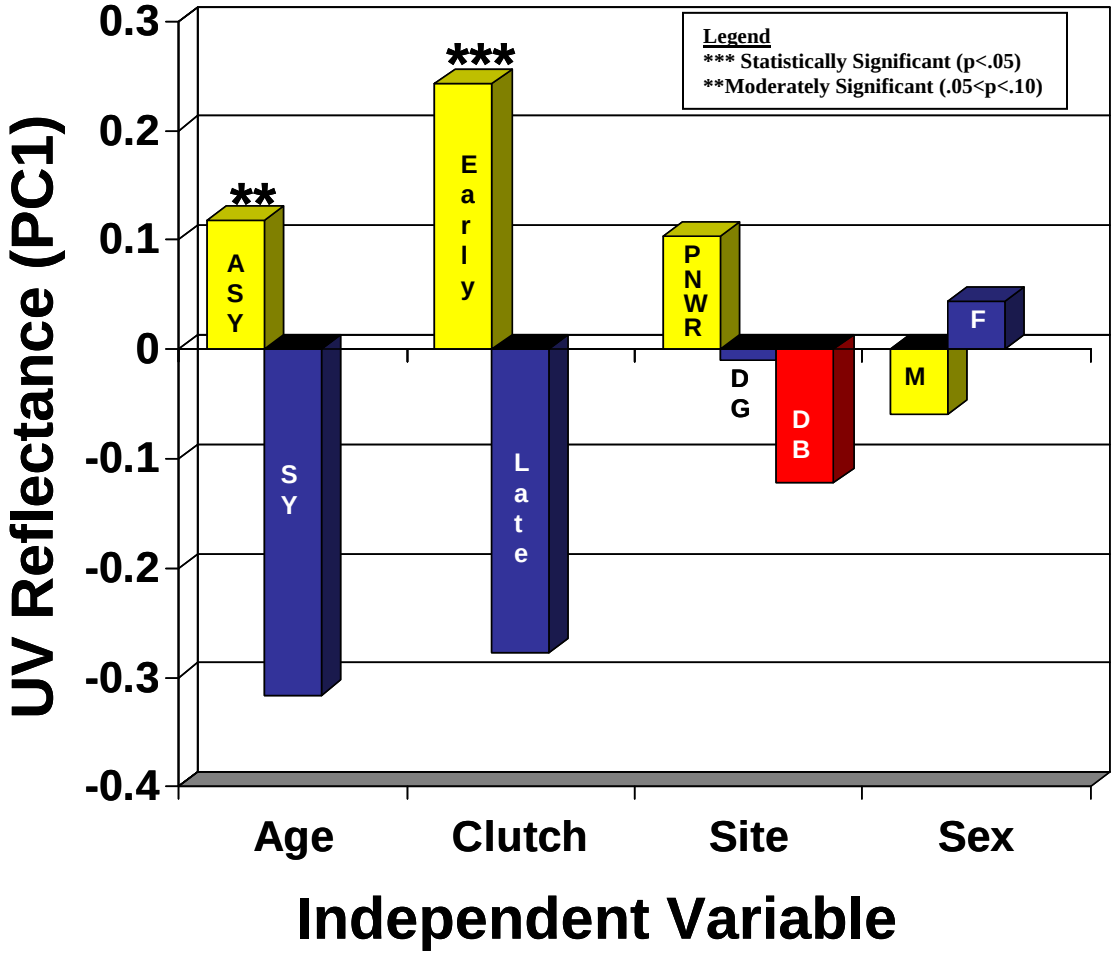


Figure 14: Mean PC2 values (representing visible light reflectance) for 2008 independent variables: age, clutch, site, and sex.

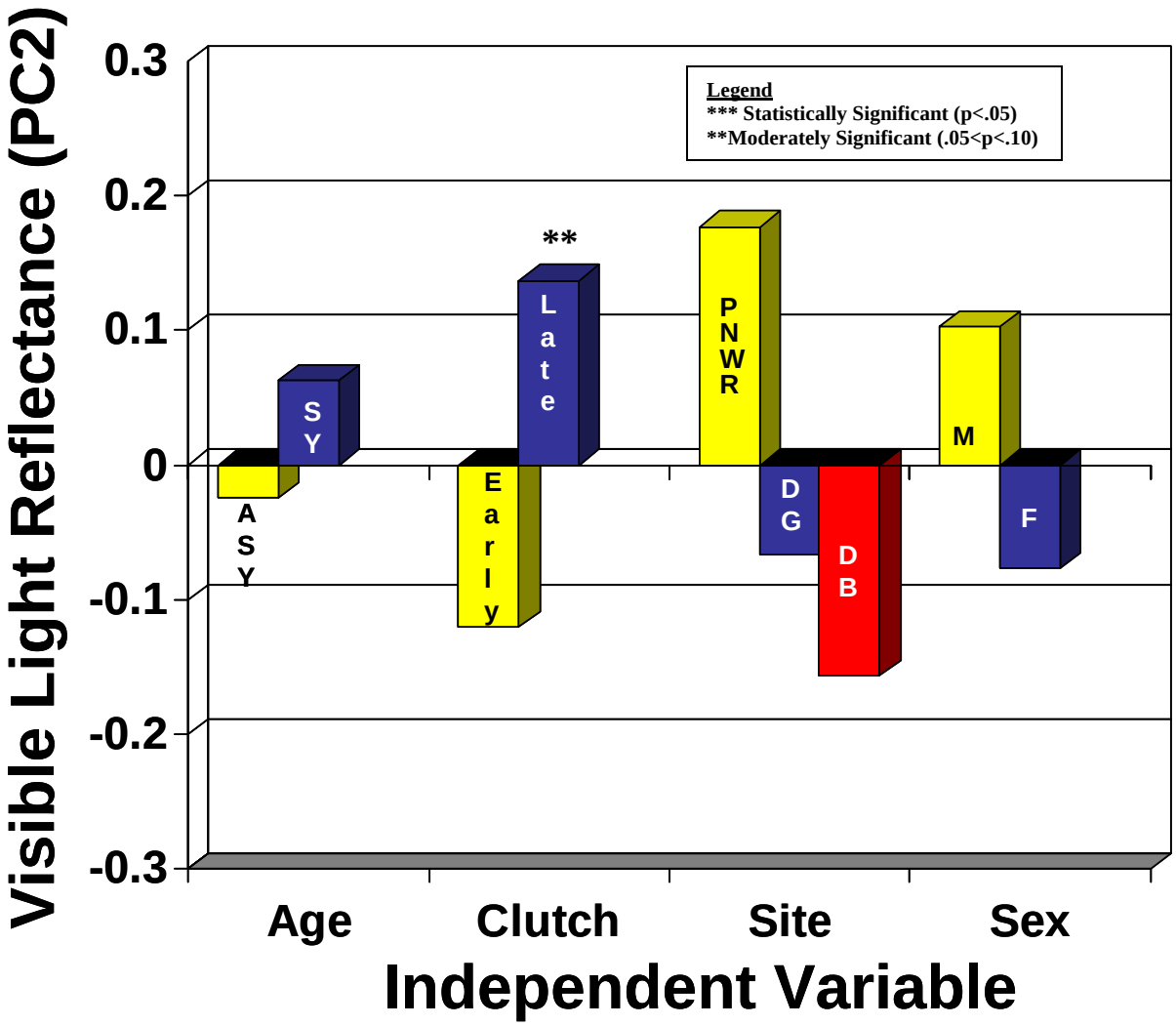


Figure 15: Mean PC3 values (representing Hue) for 2008 independent variables: age, clutch, site, and sex.

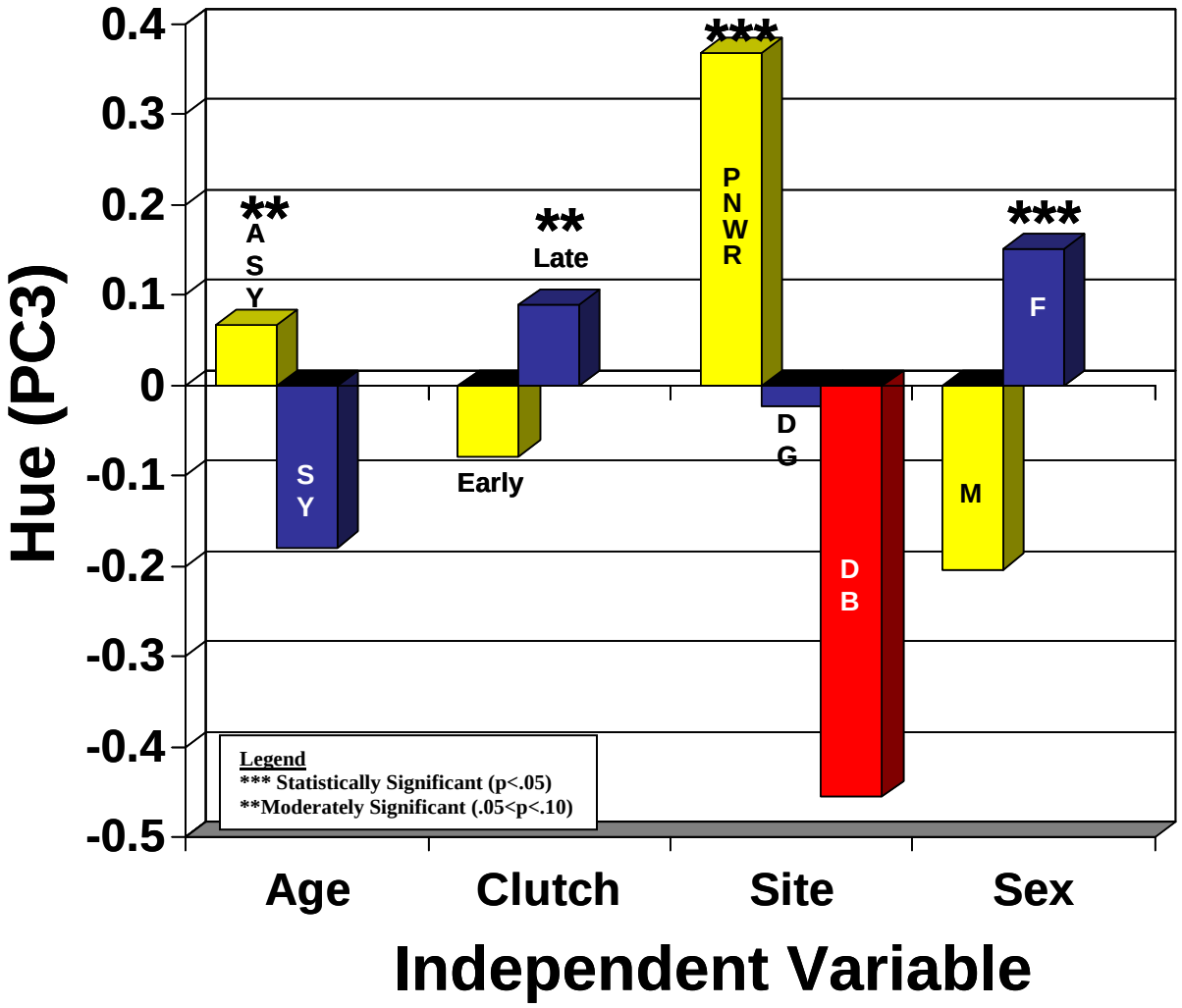
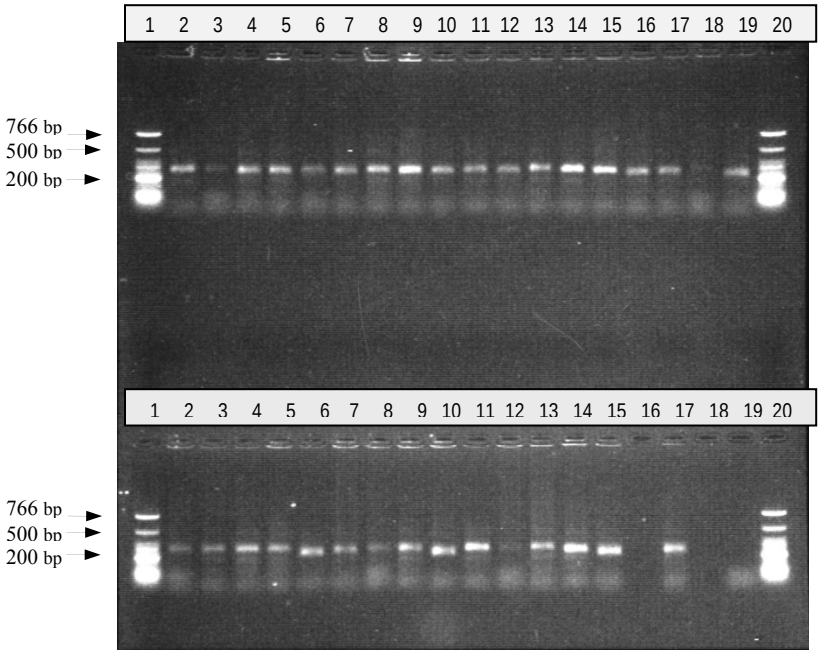


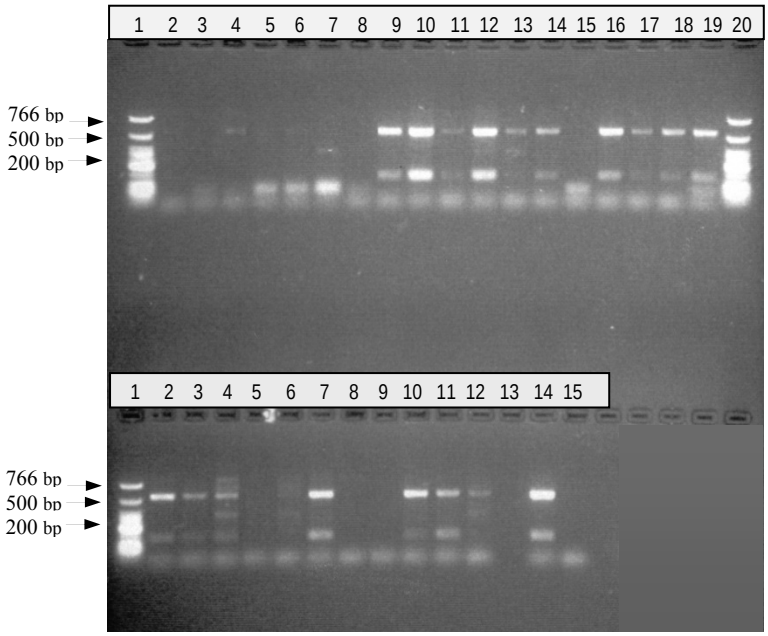
Figure 16: Gel product for Electrophoresis of a conserved region of the cytochrome b gene in the mitochondrial DNA of Prothonotary Warblers sampled on their breeding grounds in 2008. Successful amplification of the warbler's DNA was determined by the presence of a DNA fragment of approximately 280 base pairs. The list below states the bird band number from each sample shown here, as well as the two controls. Low molecular weight DNA markers were used to identify the length of DNA fragments.



TOP ROW			
Lane	**Band No.**	**Lane**	**Band No.**
1	DNA Marker	11	2500-46249
2	2350-11090	12	2360-67173
3	2500-46469	13	2450-33004
4	2500-46360	14	2430-20115
5	2460-19902	15	2250-49496
6	2540-05502	16	2450-33003
7	2540-05602	17	2450-33452
8	2540-05501	18	2350-11091
9	2500-46430	19	2450-33693
10	2500-46287	20	DNA Marker

BOTTOM ROW			
Lane	**Band No.**	**Lane**	**Band No.**
1	DNA Marker	11	2540-20229
2	2450-33096	12	2500-15902
3	2360-11680	13	2500-15907
4	2430-20321	14	2500-15743
5	2360-11431	15	2440-79405
6	2500-15368	16	– – – – – –
7	2440-80159	17	Positive Control
8	2460-19985	18	– – – – – –
9	2440-79528	19	Negative Control
10	2540-20292	20	DNA Marker

Figure 17: Gel product for Electrophoresis of the cytochrome b gene from Haemosporidia mtDNA found in the blood samples of breeding Prothonotary Warblers in 2008. The presence of absence of a DNA fragment of approximately 520 base pairs in length indicates the presence or absence of the Haemosporidia parasite. The list below states the bird band number from each sample shown here, as well as the two controls. Low molecular weight DNA markers were used to identify the length of DNA fragments.



TOP ROW			
Lane	Band No.	Lane	Band No.
1	DNA Marker	11	2500-46766
2	2440-79720	12	2540-05505
3	2280-97486	13	2500-46210
4	2200-92494	14	2500-46290
5	2500-15283	15	2450-33203
6	2440-79740	16	2450-33014
7	2360-11595	17	2500-46748
8	2540-19939	18	2450-32910
9	2500-46397	19	2350-11092
10	2500-46201	20	DNA Marker

BOTTOM ROW			
Lane	Band No.	Lane	Band No.
1	DNA Marker	11	2400-47630
2	2350-11195	12	2440-79228
3	2360-67173	13	2540-70607
4	2350-11091	14	Positive Control
5	2450-33119	15	Negative Control
6	2440-79443		
7	2440-79571		
8	2540-20546		
9	2540-20552		
10	2440-79179		

Figure 18: Mean PC1 values (representing UV reflectance) for clutch and 2008 Haemosporidia infection

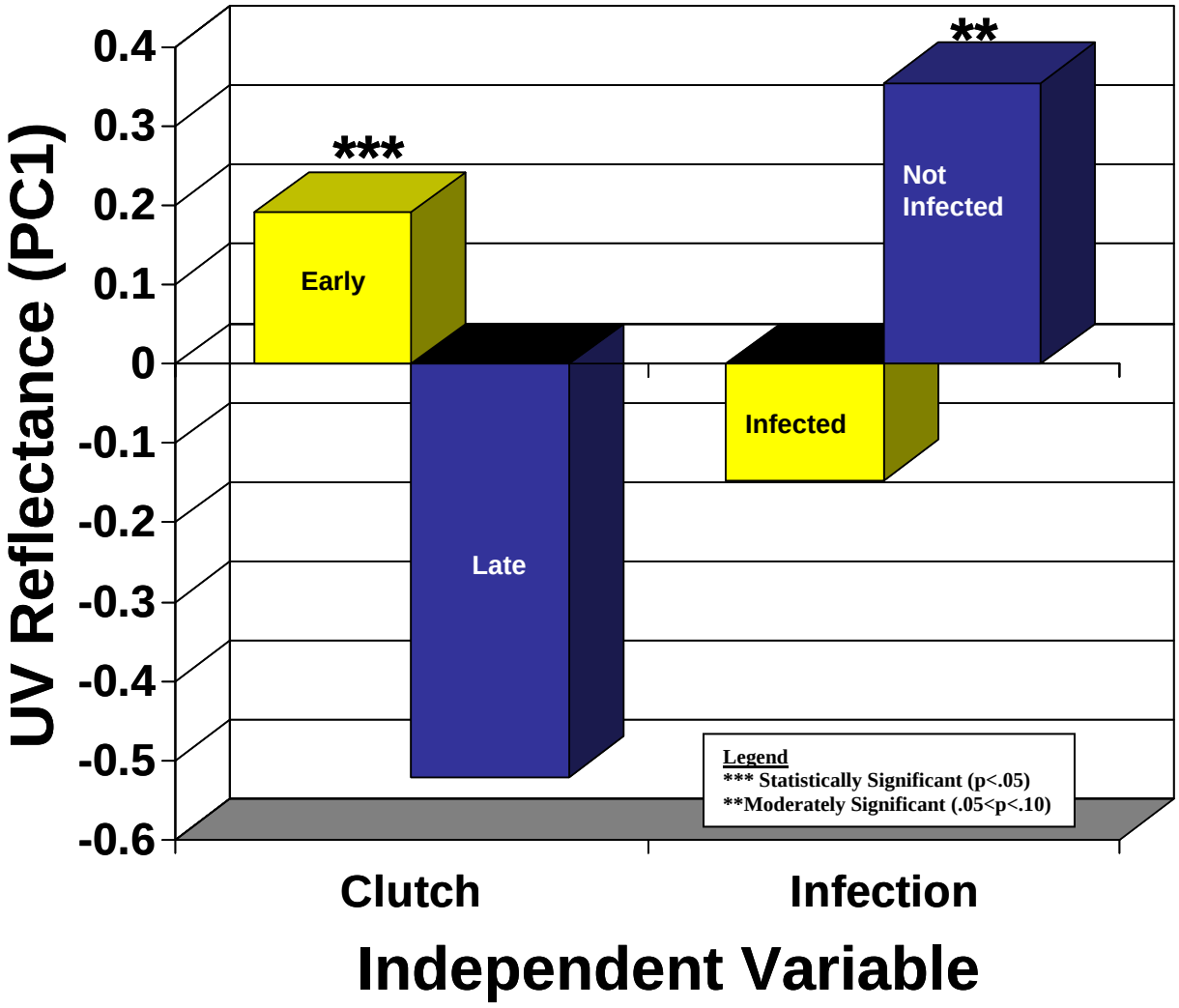


Figure 19: Mean PC2 values (representing visible light reflectance) for clutch and 2008 Haemosporidia infection

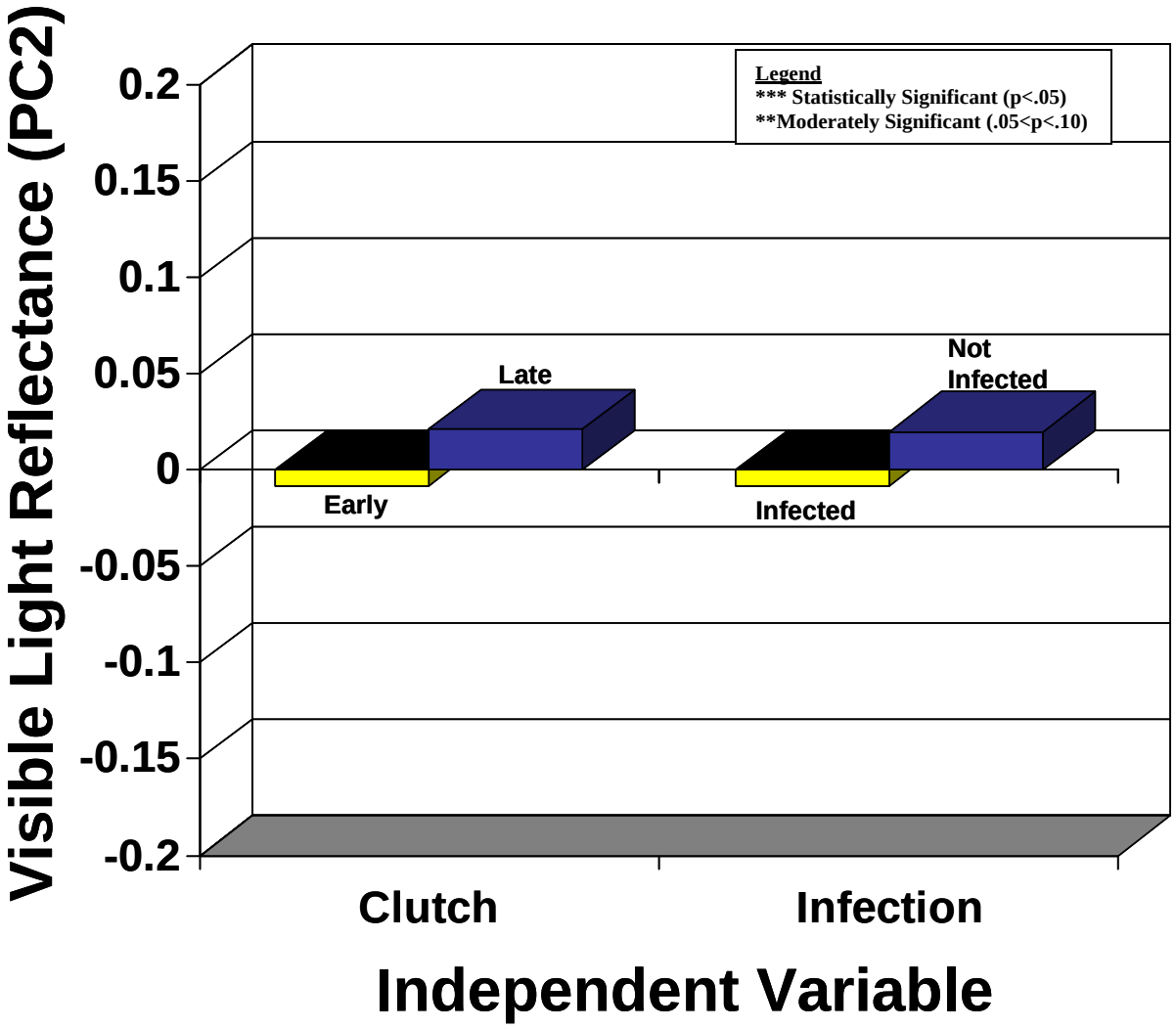
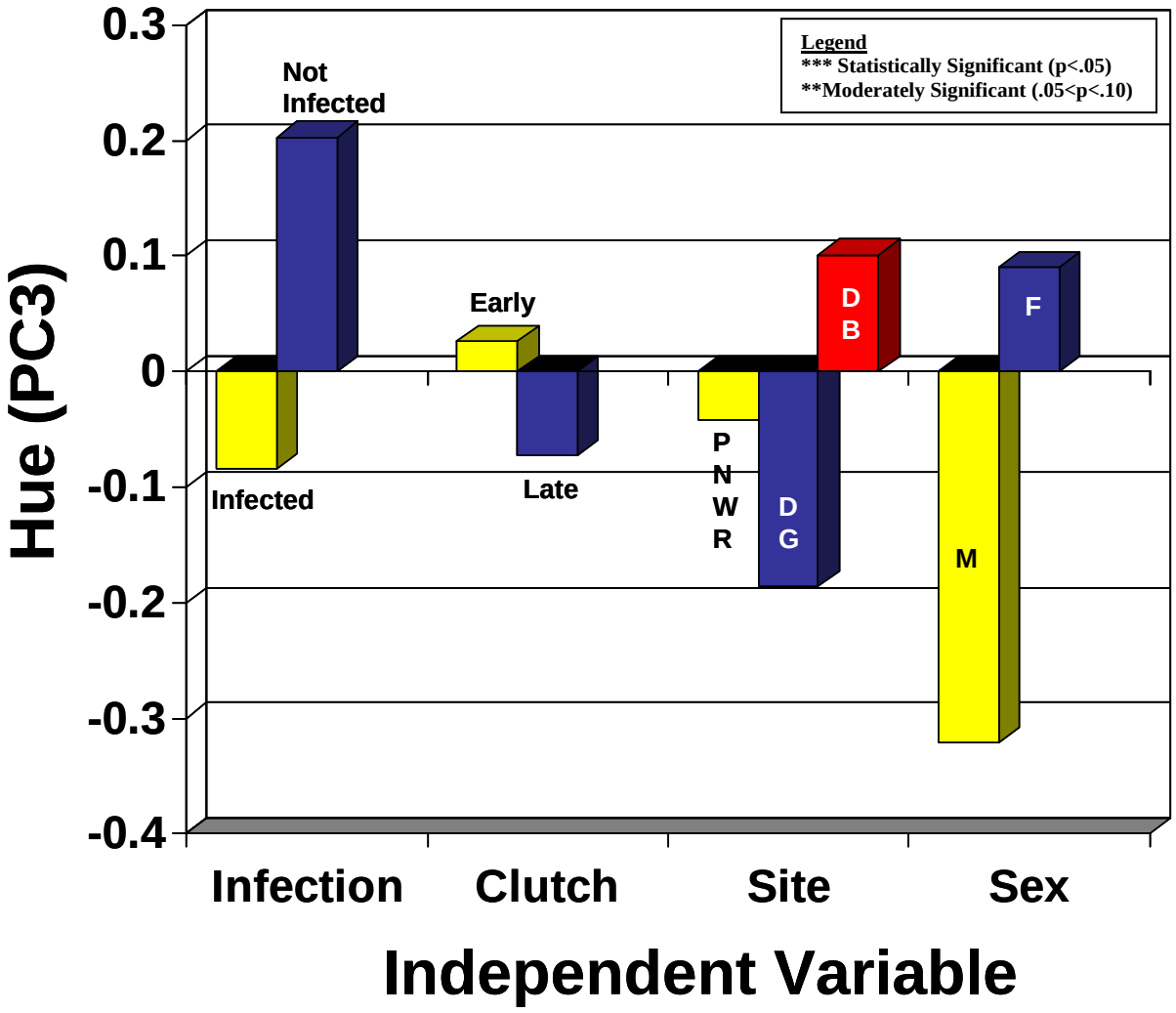


Figure 20: Mean PC3 values (representing Hue) for clutch, site, age, and 2008 Haemosporidia infection.



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Appendices

Appendix I: 2008 Prothonotary Warbler Samples

Date	Clutch	Site	Sex	Age	Mass (g)	Band #	Haem. Inf.	Intensity	Brightness	UV Brightness	UV Chroma	UV Inten-sity	Hue
4/13/2008	1	DG	M	SY		2500-46249	N	28.96	15778.44	392.93	0.0249	3.42	601.9
4/19/2008	1	DG	M	SY		2500-46242	P	26.88	16742.35	1380.73	0.08247	9.43	628.9
4/19/2008	1	DG	M	ASY		2450-32909	N	28.5	17430.43	1317.23	0.07557	9.45	629.7
5/1/2008	1	DG	M	ASY	18	2360-67117	N	26.26	15751.21	863.137	0.0548	6.58	621.6
5/1/2008	1	DG	M	ASY	19	2350-11368	P	37.5	24972.32	2947.04	0.11801	17.5	640.8
5/1/2008	1	DG	M	ASY	20	2450-33601	N	24.32	15427.40	1614.73	0.10467	10.6	611.8
5/1/2008	1	DG	M	ASY	19	2450-33434	N	32.74	18634.84	616.49	0.03308	5.44	645.8
5/4/2008	1	DG	M	SY	13.4	2500-46454	P	28.78	14442.89	36.78	0.00255	0.81	596.4
5/4/2008	1	DG	M	SY	13	2500-46475	P	25.48	12740.95	4.39	0.00034	0.25	596.2
5/4/2008	1	DG	M	ASY	13.2	2500-15966	P	29.9	15285.63	0	0	0	626.3
5/7/2008	1	DG	M	SY	13.5	2500-46305	P	33.14	19639.92	1037.57	0.05283	6.28	600.2
5/17/2008	1	DG	M	ASY	13.8	2540-05602	P	28.78	15302.58	124.69	0.00815	1.24	600.2
5/17/2008	1	DG	M	ASY	13.9	2450-32909	P	25.16	13163.05	97.63	0.00742	1.04	599.9
5/17/2008	1	DG	M	ASY	14.2	2500-46241	N	31.56	18107.44	1029.43	0.05685	6.61	643
5/23/2008	1	DG	M	SY	13.8	2500-46249	N	28.96	15778.44	392.93	0.0249	3.42	601.9
5/7/2008	1	DG	F	ASY		2360-67173	N	21.22	11896.56	491.23	0.04129	3.78	643
5/7/2008	1	DG	F	ASY		2350-11195	N	31	15468.73	23.05	0.00149	0.9	603.6
5/11/2008	1	DG	F	ASY	14.1	2450-33153	N	21.18	12902.21	1171.54	0.0908	7.82	643
5/11/2008	1	DG	F	ASY	15.4	2500-46353	N	25.52	14950.31	556.94	0.03725	3.51	585.1
5/11/2008	1	DG	F	ASY	16.5	2250-49496	N	29.78	19497.01	2015.08	0.10335	11.6	624.3
5/11/2008	1	DG	F	SY	16.3	2450-33072	N	29.76	18993.52	1855.48	0.09769	11.1	637.9
5/11/2008	1	DG	F	ASY	15.5	2360-67169	P	30.66	17517.03	1370.36	0.07823	9.05	644
5/11/2008	1	DG	F	ASY	16.3	2450-33003	N	24.62	15387.55	1475.98	0.09592	9.69	620.6
5/11/2008	1	DG	F	ASY		2450-33452	N	31.86	19033.05	1118.10	0.05875	7.79	628.9

5/11/2008	1	DG	F	ASY	15.7	2350-11091	N	32.2	20578.01	1968.37	0.09565	11.9	622.3
5/11/2008	1	DG	F	ASY	14.7	2450-33096	N	29.98	20375.46	2464.36	0.12095	13.8	629.1
5/11/2008	1	DG	F	SY	16.1	2500-46348	N	27.06	15144.68	478.18	0.03157	3.55	643.3
5/11/2008	1	DG	F	ASY	17	2360-11680	P	30.04	17708.07	956.46	0.05401	7.1	628.9
5/11/2008	1	DG	F	ASY	14.8	2450-33414	N	34.08	21496.38	1823.53	0.08483	11.1	652
5/17/2008	1	DG	F	ASY	16.4	2430-20321	P	34.18	21107.67	1995.16	0.09452	12.1	643.8
5/17/2008	1	DG	F	ASY	14.4	2450-33673	P	34.24	23969.79	2723.56	0.11362	15.3	622.4
4/18/2008	1	DB	M	ASY		2320-33623	N	27.64	16670.85	1202.74	0.07215	7.84	603.4
4/18/2008	1	DB	M	ASY		2280-97483	P	37.6	28537.44	3787.10	0.13271	20.8	596.4
4/27/2008	1	DB	M	ASY		2440-80059	P	28.24	16734.82	1096.35	0.06551	7.12	603.2
4/27/2008	1	DB	M	ASY		2360-11431	P	42.6	30075.28	3523.22	0.11715	19.8	603.6
5/14/2008	1	DB	M	ASY	14	2500-15371	P	40.9	22758.18	626.34	0.02752	5.01	625.9
5/14/2008	1	DB	M	ASY	14	2500-15372	P	24.5	12722.33	280.51	0.02205	2.12	603.2
5/14/2008	1	DB	M	ASY	14.4	2500-15373	P	39.86	22927.09	1144.82	0.04993	7.22	603.2
5/19/2008	1	DB	M	ASY	15.5	2440-79884	P	38.06	22177.29	1008.10	0.04546	7.86	624.3
5/19/2008	1	DB	M	ASY	13.6	2280-97181	P	33.3	18499.58	455.28	0.02461	3.57	624.3
5/19/2008	1	DB	M	ASY	15	2360-11581	P	28.16	14354.93	19.46	0.00136	0.34	624.7
5/22/2008	1	DB	M	ASY	13.3	2500-15375	N	25.84	12279.12	8.52	0.00069	0.38	602.2
5/22/2008	1	DB	M	ASY	15.1	2400-47222	P	31.48	15818.43	342.46	0.02165	2.76	601.9
5/3/2008	1	DB	F	SY	15.4	2500-15366	P	27.82	13772.33	7.05	0.00051	0.34	601.9
5/9/2008	1	DB	F	ASY	16.5	2400-46923	P	20.72	11759.73	289.51	0.02462	2.61	640.5
5/9/2008	1	DB	F	ASY	17.8	2360-11759	P	34.12	21539.39	2097.32	0.09737	13.4	633.2
5/9/2008	1	DB	F	SY	16.7	2500-15016	P	29.68	17765.71	979.32	0.05512	7.07	597.9
5/9/2008	1	DB	F	ASY	15.3	2400-47510	N	27.8	16066.77	718.43	0.04472	5.24	627.6
5/10/2008	1	DB	F	ASY	15.4	2440-79720	N	17.3	7344.36	0	0	0	571.5
5/10/2008	1	DB	F	SY	15.3	2460-19985	P	25.12	11836.25	0	0	0	602.1
5/10/2008	1	DB	F	SY	16	2500-15283	P	33.18	18668.71	897.57	0.04808	6.71	604.2
5/10/2008	1	DB	F	ASY	15.6	2500-15381	P	28.8	15944.10	764.88	0.04797	5.51	604.2
5/18/2008	1	DB	F	ASY	14.8	2400-47430	P	25.76	14508.43	476.05	0.03281	3.87	624.8
5/18/2008	1	DB	F	ASY	16.4	2200-92487	P	20.06	11214.05	119.99	0.0107	1	638.4
5/18/2008	1	DB	F	ASY	14.9	2400-47873	P	22.62	12880.95	314.57	0.02442	2.79	640.2
5/19/2008	1	DB	F	ASY	16.6	2500-15029	P	27.52	14124.79	181.65	0.01286	2.38	559.3
4/17/2008	1	PN WR	M	ASY		2540-20201	P	35.4	20644.51	1007.43	0.0488	7.33	633.1
5/2/2008	1	PN WR	M	ASY	14.4	2360-11085	N	37.74	23462.77	2134.14	0.09096	15	618.6

5/2/2008	1	PN WR	M	ASY	14.2	2540-20203	P	32.86	19186.31	1154.38	0.06017	9.14	641.2
5/2/2008	1	PN WR	M	ASY	14.6	2540-20225	P	28.78	17806.71	1532.18	0.08605	10.4	628.4
5/5/2008	1	PN WR	M	ASY	14.4	2540-20227	N	34.84	21002.85	1664.08	0.07923	11.9	622.9
5/5/2008	1	PN WR	M	ASY	14.4	2540-20226	P	24.86	13637.06	442.33	0.03244	3.98	644.7
5/6/2008	1	PN WR	M	ASY	15.8	2400-47633	N	18.84	10061.62	52.73	0.00524	0.59	628.9
5/6/2008	1	PN WR	M	ASY	14.1	2440-79433	P	26.48	16100.69	1192.51	0.07407	7.97	622.3
5/6/2008	1	PN WR	M	SY	15.3	2440-79652	N	27.92	16320.02	794.33	0.04867	5.63	622.3
5/15/2008	1	PN WR	M	ASY	14.2	2540-20235	N	30.3	17178.38	784.13	0.04565	6.34	621.9
5/15/2008	1	PN WR	M	ASY	14.7	2540-20231	N	25.7	14534.51	588.52	0.04049	4.87	645.8
5/15/2008	1	PN WR	M	ASY	14.4	2540-20234	P	25.34	14038.10	416.99	0.0297	3.74	622.3
5/15/2008	1	PN WR	M	ASY	14.8	2540-20233	P	34.6	21389.58	1878.13	0.08781	12.6	622.3
5/15/2008	1	PN WR	M	SY	13.9	2540-20232	N	25.72	14752.92	648.31	0.04395	4.98	618.6
5/20/2008	1	PN WR	M	SY	15.8	2440-79528	P	28.84	16844.79	757.45	0.04497	5.27	634.9
5/20/2008	1	PN WR	M	ASY	14.5	2540-20236	P	25.48	14104.31	273.85	0.01942	2.86	643.2
5/27/2008	1	PN WR	M	ASY	14.4	2540-20292	P	27.36	15519.93	592.14	0.03815	4.67	622.5
5/27/2008	1	PN WR	M	ASY	14.3	2440-79175	P	31.8	18675.91	1039.78	0.05568	7.98	623.9
5/5/2008	1	PN WR	F	ASY	15.1	2440-79013	P	42.32	24621.23	1034.31	0.04201	6.58	643.2
5/13/2008	1	PN WR	F	ASY	16.1	2500-15903	P	32.36	18928.87	977.60	0.05165	7.44	642.5
5/13/2008	1	PN WR	F	ASY	15.9	2500-15902	P	33.08	19750.44	1220.84	0.06181	8.43	644.9
5/13/2008	1	PN WR	F	SY	16.4	2500-15880	P	34.2	20540.18	1113.93	0.05423	8.39	643.5
5/13/2008	1	PN WR	F	ASY		2500-15905	P	29.32	17362.25	691.13	0.03981	5.03	585.7
5/13/2008	1	PN WR	F	ASY		2500-15907	N	27.92	15483.45	351.78	0.02272	3.77	602.1
5/13/2008	1	PN WR	F	ASY	16.6	2500-15909	N	30.98	19148.31	1665.87	0.087	11.4	643
5/13/2008	1	PN WR	F	ASY	17	2440-79444	N	43	25451.05	1076.83	0.04231	7.31	655
5/13/2008	1	PN WR	F	ASY		2440-79457	N	25.66	13619.82	52.84	0.00388	0.76	601.9
5/13/2008	1	PN WR	F	ASY		2440-79503	P	18.76	8989.95	81.62	0.00908	1.01	602.5
5/13/2008	1	PN WR	F	ASY	16.4	2540-20230	P	30.64	18469.18	1349.50	0.07307	9.24	648.4
5/13/2008	1	PN WR	F	ASY	15.2	2440-79405	P	28.12	16168.60	942.67	0.0583	7.05	664.2
5/13/2008	1	PN WR	F	ASY		2400-47147	P	33.08	19353.75	1166.32	0.06026	8.75	639.5
5/13/2008	1	PN WR	F	ASY	15	2540-20207	P	26.1	14779.79	618.10	0.04182	4.32	641.5
5/15/2008	1	PN WR	F	ASY	16.7	2440-79041	P	18.68	10589.23	239.256	0.02259	2.01	622.9
5/20/2008	1	PN WR	F	SY	16.2	2500-15671	P	26.08	16722.74	1483.95	0.08874	9.34	645.8
7/4/2008	2	DB	F	ASY		2430-20308	P	23.08	11157.88	75.13	0.00673	1.04	603.2
6/18/2008	2	DB	F	ASY		2350-11331	N	27.08	17351.84	1172.07	0.06755	7.26	602.9

6/18/2008	2	DB	F	ASY	14.8	2440-80169	P	24.92	14031.14	315.20	0.02246	2.8	640.7
6/18/2008	2	DB	F	ASY	16.3	2400-46922	P	27.28	15794.73	593.56	0.03758	4.49	643.2
6/18/2008	2	DB	F	ASY	15.8	2440-80006	P	28.36	16796.20	1055.72	0.06285	7.11	645.9
6/18/2008	2	DB	F	ASY	14.8	2500-15381	P	28.8	15944.10	764.88	0.04797	5.51	604.2
6/18/2008	2	DB	F	ASY	16.2	2200-92494	P	39.18	23859.94	1121.62	0.04701	7.26	622.3
6/18/2008	2	DB	F	SY	16.4	2500-15180	P	30.86	17585.33	466.05	0.0265	3.8	622.3
6/18/2008	2	DB	F	SY	14.7	2500-15283	P	26.36	14931.17	316.95	0.02123	2.07	628.9
6/26/2008	2	DB	F	ASY		2540-19935	P	20.46	11383.00	235.51	0.02069	2.27	635.6
6/26/2008	2	DB	F	ASY	14.8	2400-47078	P	32.3	18924.53	803.70	0.04247	5.61	641.5
6/26/2008	2	DB	F	ASY	14.1	2500-15333	N	29.46	18207.76	1287.36	0.0707	8.27	603.2
6/26/2008	2	DB	F	ASY	15.5	2360-11759	N	34.12	21539.39	2097.30	0.09737	13.4	633.2
6/26/2008	2	DB	F	ASY	16	2360-11091	P	31.62	18459.94	685.91	0.03716	5.66	622.3
6/26/2008	2	DB	F	SY		2540-19936	N	28.08	15196.13	18.26	0.0012	0.47	603.2
6/26/2008	2	DB	F	ASY	15.6	2440-80168	P	24.298	13895.79	515.90	0.03713	4.13	603.6
6/26/2008	2	DB	F	SY	14.7	2460-19985	N	25.12	11836.25	0	0	0	602.1
6/26/2008	2	DB	F	ASY	14.1	2500-15081	N	23.12	13751.03	789.65	0.05742	4.92	640.5
6/26/2008	2	DB	F	SY	14.2	2500-15395	N	29.78	17174.46	477.69	0.02781	4.89	603.2
7/7/2008	2	DB	F	SY		2540-19810	N	19.8	8420.84	0	0	0	603.2
7/8/2008	2	DG	F	ASY		2500-46397	P	24.2	14046.71	813.52	0.05792	6	628.9
6/19/2008	2	DG	F	SY	14.8	2500-46201	P	32.24	19691.69	1298.33	0.06593	8.54	641
6/19/2008	2	DG	F	SY	15.2	2500-46766	P	33.56	20542.59	852.22	0.04149	5.02	640.8
6/19/2008	2	DG	F	ASY	15.3	2540-05505	P	29.36	17302.63	600.29	0.03469	4.65	641.7
6/19/2008	2	DG	F	SY		2500-46210	P	33.6	19991.39	782.15	0.03912	5.48	640.8
6/19/2008	2	DG	F	SY	15.5	2500-46290	P	27.78	16176.42	647.56	0.04003	4.89	637.4
6/19/2008	2	DG	F	SY		2450-33014	P	27.62	17226.96	1156.59	0.06714	7.42	628.9
6/16/2008	2	DG	F	SY	15.2	2500-46748	P	15.62	7634.06	0	0	0	604.2
6/16/2008	2	DG	F	ASY	14.9	2450-32910	P	34.08	18371.23	173.70	0.00946	1.71	626.6
6/8/2008	2	DG	F	ASY		2350-11092	P	24.52	12535.14	0	0	0	600.2
7/2/2008	2	DG	F	ASY		2350-11195	P	31	15468.73	23.05	0.00149	0.9	603.6
7/2/2008	2	DG	F	ASY		2360-67173	P	21.22	11896.56	491.23	0.04129	3.78	643
7/2/2008	2	DG	F	ASY		2350-11091	P	31.62	18459.94	685.91	0.03716	5.66	622.3
6/27/2008	2	DG	F	SY		2450-33119	N	36.52	19920.97	172.24	0.00865	2.51	636.9
6/11/2008	2	PN WR	F	SY	14.6	2540-20546	N	38.1	26232.92	2839.75	0.10825	15.8	621.6
6/11/2008	2	PN WR	F	ASY		2440-79179	P	30.36	18126.60	1067.27	0.05888	7.25	624.3

6/10/2008	2	PN WR	F	ASY	14.0	2400-47630	P	26.34	15560.45	850.45	0.05465	5.78	621.6
6/10/2008	2	PN WR	F	ASY	15.2	2440-79237	P	35.84	22015.27	1530.38	0.06951	9.61	633.2
6/17/2008	2	PN WR	F	SY	16.5	2540-20611	P	30.22	16660.99	231.60	0.0139	2.35	624.3
6/10/2008	2	PN WR	F	ASY	13.9	2440-79208	P	38.16	22566.42	1238.54	0.05488	8.83	654.8
6/17/2008	2	PN WR	F	SY		2540-20219	N	31.2	17893.84	679.30	0.03796	3.92	624.3
6/30/2008	2	PN WR	F	SY	14.9	2500-46364	P	28.9	16645.25	543.04	0.03262	4.21	633.9
6/24/2008	2	PN WR	F	ASY	14.1	2360-11734	P	30.7	19251.14	1235.48	0.06418	7.89	624.4
6/24/2008	2	PN WR	F	ASY		2440-79013	N	42.32	24621.23	1034.31	0.04201	6.58	643.2
6/24/2008	2	PN WR	F	SY	14.1	2500-15962	N	33.8	19981.97	1089.51	0.05452	7.59	644.7
6/24/2008	2	PN WR	F	ASY	14.9	2440-79053	N	36.32	23230.27	2185.63	0.09409	12.7	642.5
6/24/2008	2	PN WR	F	ASY	14.1	2440-79444	N	43	25451.05	1076.83	0.04231	7.31	655
6/25/2008	2	PN WR	F	ASY	16.5	2440-79041	P	28.42	16392.06	453.72	0.02768	3	603.6
6/25/2008	2	PN WR	F	ASY	13.2	2440-79114	P	32.68	19099.33	613.54	0.03212	4.57	634.2
6/25/2008	2	PN WR	F	ASY	14.1	2360-11657	N	33.54	19393.11	875.77	0.04516	5.54	633.6
6/25/2008	2	PN WR	F	ASY	15.4	2440-79539	P	32.62	18382.93	399.59	0.02174	2.82	634.1
6/25/2008	2	PN WR	F	ASY	13.2	2430-20488	P	32.24	19156.28	818.21	0.04271	6.12	624.3
7/1/2008	2	PN WR	M	ASY	13.2	2440-79030	N	33.26	20819.52	1662.87	0.07987	10.2	634.6
6/11/2008	2	PN WR	M	ASY		2540-20632	P	25.5	14307.19	220.85	0.01544	2.32	625.1
6/11/2008	2	PN WR	M	SY	14	2540-20633	P	21.06	11812.51	333.93	0.02827	2.93	626.8
6/11/2008	2	PN WR	M	ASY	14.2	2540-20201	N	35.4	20644.51	1007.43	0.0488	7.33	633.1
6/17/2008	2	PN WR	M	SY	14.2	2540-20634	P	32.04	17420.98	119.18	0.00684	1.49	624.3
6/17/2008	2	PN WR	M	SY	13.6	2500-15638	P	33.12	19315.44	643.47	0.03331	4.35	624.3
6/11/2008	2	PN WR	M	ASY	13.6	2540-20608	P	27.74	17095.68	1523.84	0.08914	9.33	612.3
6/11/2008	2	PN WR	M	ASY	13.5	2540-20610	P	28.14	16496.24	1038.11	0.06293	7.69	628.9
6/11/2008	2	PN WR	M	ASY	13.9	2500-15979	P	28.18	17868.60	1510.21	0.08452	9.73	620.3
6/17/2008	2	PN WR	M	SY	13.1	2540-20612	N	35.64	20256.86	637.65	0.03148	5.48	624.3
6/17/2008	2	PN WR	M	SY	14.1	2500-15241	P	35.52	22162.93	1907.74	0.08608	12.6	639.5
6/30/2008	2	PN WR	M	ASY		2540-20627	P	31.18	16658.08	137.82	0.00827	1.52	636.5
7/4/2008	2	DB	M	ASY	13.4	2540-21140	P	33.4	17095.44	207.28	0.01213	3	603.2
6/26/2008	2	DB	M	ASY		2500-15378	P	27.8	15427.13	353.74	0.02293	2.99	603.6
6/23/2008	2	DB	M	ASY		2500-15376	P	21.06	11593.25	146.74	0.01266	1.78	621.3
6/23/2008	2	DB	M	ASY		2500-15377	P	23.3	13259.46	325.33	0.02454	2.87	633.6
7/8/2008	2	DG	M	ASY		2540-05602	P	30.42	17216.59	373.98	0.02172	2.95	643.6
7/8/2008	2	DG	M	SY		2500-46427	P	27.24	13497.20	0	0	0	603.6

7/8/2008	2	DG	M	ASY		2450-33649	P	26.58	12810.8	0	0	0	603.4
6/22/2008	2	DG	M	ASY		2350-11090	P	30.46	16705.67	268.38	0.01607	2.13	626.6
6/13/2008	2	DG	M	SY	13.2	2500-46469	P	35.64	19354.84	521.26	0.02693	5.14	603.6
6/13/2008	2	DG	M	SY	13.9	2500-46360	P	24.6	11902.74	10.33	0.00087	0.4	603.9
6/13/2008	2	DG	M	SY	14.1	2540-05502	P	35.36	19717.48	489.26	0.02481	3.89	603.6
6/12/2008	2	DG	M	ASY	13.9	2540-05602	P	34.06	18085.10	228.09	0.01261	2.07	626.6
6/9/2008	2	DG	M	ASY	14.8	2540-05501	P	32.1	18600.25	753.37	0.0405	6.12	623.3
6/9/2008	2	DG	M	SY	13.9	2500-46430	P	35.4	19250.26	342.80	0.01781	4.53	601.9
6/19/2008	2	DG	M	SY	13.3	2500-46287	P	27.72	14358.53	15.33	0.00107	0.34	626.6

Appendix II: Data on Recaptured birds.

Sample	sex	date	band #	infection	wnv	slev	plas	haem
1	M	4/22/2009	2500-46430	Y	Y	N	Y	N
2	F	5/5/2009	2360-11734	Y			Y	Y
3	M	5/6/2009	2440-80059	Y			Y	Y
4	M	5/6/2009	2500-15376	Y				
5	F	5/11/2009	2440-79571	Y			Y	Y
6	F	5/11/2009	2440-79539	Y			Y	N
7	F	5/12/2009	2400-47630	Y			Y	N
8	F	5/12/2009	2440-79114	Y			Y	Y
9	F	5/12/2009	2400-47147	Y			Y	N
10	F	5/12/2009	2440-79503	Y			Y	N
11	F	5/13/2009	2400-46922	Y			Y	Y
12	F	5/13/2009	2500-15180	Y	Y	N	Y	N
13	F	5/13/2009	2500-15069	Y			Y	N
14	F	5/13/2009	2440-80006	Y				
15	F	5/14/2009	2440-80169	Y	Y	N	Y	Y
16	F	5/14/2009	2540-19935	Y			Y	Y
17	F	5/18/2009	2400-46923	Y				
18	M	5/19/2009	2450-33649	Y			Y	Y
19	M	5/19/2009	2540-05502	Y	N	N		
20	F	5/28/2009	2500-15283	Y			Y	Y
21	M	5/29/2009	2540-20201	Y			N	Y
22	F	5/29/2009	2440-79208	Y	Y	Y	N	Y
23	F	6/11/2009	2360-11091	Y			Y	N
24	F	6/11/2009	2500-15369	Y			Y	Y
25	F	6/16/2009	2360-67169	Y				
26	F	6/16/2009	2400-47430	Y			Y	N
27	F	6/16/2009	2440-80168	Y			Y	Y
28	F	6/16/2009	2320-33691	Y			Y	N
29	F	6/19/2009	2450-33673	Y				
30	F	6/19/2009	2450-33452	N				
31	F	6/19/2009	2430-20114	N				
32	M	4/23/2009	2500-46249	N	N	N		
33	F	5/11/2009	2540-20546	N				
34	F	5/12/2009	2500-15909	N				
35	F	5/12/2009	2540-20219	N				
36	F	5/13/2009	2500-15081	N				
37	F	5/13/2009	2500-15395	N				
38	F	5/14/2009	2360-11595	N				
39	F	5/18/2009	2540-19936	N				
40	M	6/29/2009	2500-15367	N				
41	M	6/24/2009	2540-20231	N	N	N		

Sample	int08	bright08	uvbright08	uvchroma08	uvmax08	hue08
1	35.4	19250.26	342.80442	0.01780778	4.526	601.89
2	30.7	19251.15	1235.486642	0.064177297	7.89	624.435
3	28.24	16734.82	1096.353392	0.065513292	7.122	603.23
4	21.06	11593.25	146.7475017	0.012658008	1.782	621.28
5						
6	32.62	18382.94	399.59245	0.021737137	2.822	634.14
7	26.34	15560.46	850.452472	0.054654724	5.776	621.61
8	32.68	19099.33	613.54746	0.032124026	4.572	634.22
9	33.08	19353.75	1166.32004	0.060263247	8.752	639.52
10	18.76	8989.951	81.627	0.009079805	1.006	602.45
11	27.28	15794.74	593.5623	0.037579747	4.485	643.15
12	30.86	17585.33	466.057426	0.026502624	3.798	622.27
13	36.98	21910.13	1531.85972	0.069915601	10.18	604.24
14	28.36	16796.21	1055.720974	0.062854725	7.114	645.945
15	24.92	14031.14	315.208408	0.022464915	2.8028	640.675
16	20.46	11383	235.514762	0.020690036	2.272	635.55
17	20.72	11759.74	289.51194	0.024618909	2.614	640.51
18	26.58	12810.84	0	0	0	603.4
19	35.36	19717.48	489.26382	0.024813707	3.888	603.57
20	33.18	18668.71	897.57174	0.04807893	6.71	604.24
21	35.4	20644.51	1007.432782	0.048799056	7.3312	633.065
22	38.16	22566.42	1238.54896	0.054884595	8.828	654.825
23	31.62	18459.94	685.9119827	0.037156778	5.664	622.27
24	28.8	14595.4	122.33834	0.008381979	1.55	571.52
25	30.66	17517.03	1370.364162	0.078230393	9.052	643.975
26	25.76	14508.43	476.05534	0.032812324	3.872	624.77
27	24.298	13895.79	515.90208	0.037126495	4.132	603.57
28						
29	34.24	23969.79	2723.5606	0.113624697	15.31	622.44
30	31.86	19033.05	1118.103884	0.058745385	7.788	628.92
31						
32	28.96	15778.45	392.93616	0.024903349	3.422	601.89
33	38.1	26232.92	2839.752	0.10825145	15.8	621.61
34	30.98	19148.31	1665.87716	0.08699864	11.398	642.985
35	31.2	17893.84	679.304	0.037963007	3.92	624.27
36	23.12	13751.03	789.652	0.05742493	4.92	640.51
37	29.78	17174.47	477.69056	0.027813994	4.886	603.2333
38						
39	28.08	15196.14	18.265092	0.001201956	0.471	603.2333
40	36.54	21595.68	1014.91114	0.046996024	8.356	629.92
41	25.7	14534.51	588.52974	0.040491883	4.8666	645.78

Sample	int09	bright09	uvbright09	uvchroma09	uvmax09	hue09
1	34.38	20141.37	1247.26518	0.06192553	10.032	647.265
2	22.7	13545	693.21582	0.051178714	5.29	593.83
3	31.28	19018.56	1296.722124	0.068181931	9.824	611.6417
4	23.04	12986.55	296.56728	0.022836491	3.106	593.83
5	21.08	12086.44	351.0620707	0.029045942	3.47	602.7358
6	26.88	16448.13	984.742488	0.059869581	7.652	621.11
7	24.16	13282.66	72.947442	0.005491931	1.082	623.94
8	23.52	12586.12	73.428172	0.00583406	1.1098	630.58
9	24.54	13797.23	310.984228	0.02253961	2.738	643.15
10	32.16	19291.7	1007.58848	0.052229115	7.504	631.075
11	29.14	18371.77	1641.973076	0.089374809	11.498	642.49
12	27.14	16103.27	884.712838	0.054939937	6.8566	642.82
13	20.8	11792.65	363.470248	0.030821749	3.502	643.15
14	21.9	13204.57	806.82476	0.061101916	5.89284	623.105
15	22.52	12742.4	292.380091	0.022302563	2.5845	630.3888
16	23	12675.01	153.6574017	0.01212286	1.604	623.27
17	16.976	9383.219	110.359282	0.011761345	1.3458	632.07
18	30.26	16849.84	567.176	0.03366062	4.6008	642.16
19	30.6	17600.42	830.92814	0.047210706	6.6428	642.655
20	25.24	14380.03	235.44342	0.016372949	2.0446	631.24
21	31.88	17851.12	544.22034	0.030486623	4.818	630.085
22	25.18	14173.61	313.781852	0.022138464	2.966	615.61
23	25.14	14563.43	496.921568	0.034121193	4.518	622.27
24	27.32	15743.41	414.595678	0.026334557	3.7166	641.665
25	25.02	13916.63	231.89964	0.016663488	2.26	622.27
26	17.06	9260.325	136.83996	0.014777015	1.302	642.16
27	26.48	15592.02	777.965976	0.049895149	6.1366	623.05
28	26.64	15585.91	530.42442	0.034032311	4.014	626.43
29	26.82	15872.9	696.64124	0.043888729	5.622	601.22
30	24.64	14120.38	434.05476	0.0307396	3.3068	632.9343
31	26.86	15154.23	319.35116	0.021073398	2.8076	626.6
32	26.48	16090.44	1161.6342	0.072194071	8.764	593.83
33	23.9	13713.86	470.987192	0.034343872	4.194	644.14
34	31.3	17909.04	742.429836	0.041455585	5.862	642.49
35	28.96	17324.88	1203.484156	0.069465673	8.076	642.16
36	29.7	18875.96	1594.0295	0.084447594	10.984	622.77
37	26.56	16894.72	1595.13116	0.094415943	9.894	643.97
38	20.42	12087.91	612.09768	0.050637168	4.646	649.08
39	27.24	15848.66	681.132238	0.042977279	5.742	620.61
40	19.44	10629.33	63.19646	0.005945482	0.674	615.94
41	32.22	18285.08	541.30344	0.02960356	4.352	641.5

Sample	dint	dbright	duvbright	duvchroma	duvmax	dhue
1	-1.02	891.1116	904.46076	0.04411775	5.506	45.375
2	-8	-5706.15	-542.270822	-0.012998583	-2.6	-30.605
3	3.04	2283.736	200.368732	0.002668639	2.702	8.411667
4	1.98	1393.298	149.8197783	0.010178483	1.324	-27.45
5	21.08	12086.44	351.0620707	0.029045942	3.47	602.7358
6	-5.74	-1934.81	585.150038	0.038132444	4.83	-13.03
7	-2.18	-2277.8	-777.50503	-0.049162793	-4.694	2.33
8	-9.16	-6513.21	-540.119288	-0.026289966	-3.4622	-3.64
9	-8.54	-5556.52	-855.335812	-0.037723637	-6.014	3.63
10	13.4	10301.75	925.96148	0.04314931	6.498	28.625
11	1.86	2577.027	1048.410776	0.051795062	7.013	-0.66
12	-3.72	-1482.06	418.655412	0.028437313	3.0586	20.55
13	-16.18	-10117.5	-1168.389472	-0.039093852	-6.678	38.91
14	-6.46	-3591.63	-248.896214	-0.001752809	-1.22116	-22.84
15	-2.4	-1288.75	-22.828317	-0.000162351	-0.2183	-10.2863
16	2.54	1292.008	-81.85736034	-0.008567176	-0.668	-12.28
17	-3.744	-2376.52	-179.152658	-0.012857564	-1.2682	-8.44
18	3.68	4038.999	567.176	0.03366062	4.6008	38.76
19	-4.76	-2117.06	341.66432	0.022396999	2.7548	39.085
20	-7.94	-4288.69	-662.12832	-0.031705981	-4.6654	27
21	-3.52	-2793.39	-463.212442	-0.018312433	-2.5132	-2.98
22	-12.98	-8392.82	-924.767108	-0.032746132	-5.862	-39.215
23	-6.48	-3896.51	-188.9904147	-0.003035585	-1.146	0
24	-1.48	1148.007	292.257338	0.017952578	2.1666	70.145
25	-5.64	-3600.4	-1138.464522	-0.061566906	-6.792	-21.705
26	-8.7	-5248.11	-339.21538	-0.018035309	-2.57	17.39
27	2.182	1696.223	262.063896	0.012768655	2.0046	19.48
28				0.034032311		
29	-7.42	-8096.9	-2026.91936	-0.069735968	-9.688	-21.22
30	-7.22	-4912.67	-684.049124	-0.028005785	-4.4812	4.014286
31				0.021073398		
32	-2.48	311.9911	768.69804	0.047290722	5.342	-8.06
33	-14.2	-12519.1	-2368.764808	-0.073907578	-11.606	22.53
34	0.32	-1239.27	-923.447324	-0.045543055	-5.536	-0.495
35	-2.24	-568.965	524.180156	0.031502666	4.156	17.89
36	6.58	5124.93	804.3775	0.027022665	6.064	-17.74
37	-3.22	-279.746	1117.4406	0.066601949	5.008	40.73667
38				0.050637168		
39	-0.84	652.5217	662.867146	0.041775323	5.271	17.37667
40	-17.1	-10966.4	-951.71468	-0.041050543	-7.682	-13.98
41	6.52	3750.568	-47.2263	-0.010888323	-0.5146	-4.28

Vita

Robert Charles Fithian was born on November 26, 1984 in Arlington, VA. He grew up in Springfield, VA with his parents, Robert William Fithian and Wilma Fithian, and his two younger sisters, Annie and Lauren. Robert went to high school at West Springfield High School in Springfield, VA. Robert discovered an interest in science at a young age with the help of his parents who were educated in the sciences. He attended The College of William and Mary and throughout his undergraduate education, remained on the Biology tract. At William and Mary Robert worked two summers under the tutelage of Ruth Beck studying and managing several populations of breeding gulls, terns, and other shorebirds. He also attended an ornithology course with Dr. Dan Cristol where his interest in avian research continued to grow. Robert graduated with his Bachelor's of Science degree in Biology in 2007 and immediately began work on his Master's degree in Biology in the Parasitology lab of Dr. Ghislaine Mayer. Along with his research, he enjoyed aiding Dr. Lesley Bulluck for two semesters in her Ornitholgy class. Robert also taught introductory biology 101 labs and Human Anatomy 201 labs. For his ecological research he was awarded two Rice Center grants from VCU's Center for Environmental Life Sciences. In the future he hopes to continue studying and managing wildlife disease.